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The Toxicity and Carcinogenic Activity of 2-Acetaminofluorene*

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INTRODUCTION

One of the major problems confronting entomologists is the discovery of effective insecticides to replace the present and extensively used lead, arsenic and fluorine sprays, which present a public health hazard. For this reason, the Bureau of Entomology and Plant Quarantine, U. S. Department of Agriculture, is actively engaged in testing a wide variety of organic compounds for insecticidal activity. Obviously there would be no merit in substituting for the undesirable inorganic sprays other compounds equally hazardous to the public and to those individuals engaged in the production and application of the sprays or dusting materials. Therefore, as many as possible of those substances which show promise of effectiveness against insects are referred to this laboratory for investigation of acute and chronic toxicity to warm blooded animals. The importance of this cooperative program of research is particularly well illustrated by the discovery of the carcinogenic properties possessed by the insecticide 2-acetaminofluorene.

The 2-acetaminofluorene was furnished to us by the Division of Insecticide Investigations of the Bureau of Entomology and Plant Quarantine. This compound is a light tan, crystalline material. Various authors have reported melting points ranging from 187-194° C. The material used by us melts at 191° C. (corrected). The compound is insoluble in water, but soluble in the alcohols, glycols, and fat solvents. The addition of one or two volumes of water to a solution in alcohol or in one of the glycols causes precipitation. The compound has the following structural formula shown in Fig. 1.

ACUTE TOXICITY

Acetaminofluorene has little or no acute toxicity, as shown by observations on 21 rats, 7 mice, and 2 rabbits, the compound being given subcutaneously, gastrically, or by implantation of the crystalline material. Six adult male rats received 50 mgm. of acetaminofluorene per kg. of body weight. The compound was dissolved in propylene glycol and injected subcutaneously. A second injection was administered 2 weeks later to 5 of these 6 rats, employing the same dosage. There were no reactions of any kind, either immediately or at any time during the 3 months following the first injection. Five adult male rats were given acetaminofluorene in an acacia suspension by stomach tube, the dosage ranging from 50 to 1,000 mgm. per kg. of body weight. No reactions of any kind were noted during a period of 19 days. Five adult female rats received by stomach tube in similar manner 1 gm. of acetaminofluorene per kg. of body weight. One of these rats died of an acute lung infection after 8 months; the others were still in good condition at the end of the 14th month. At no time were any toxic reactions observed. This high acute dosage permitted an observation which was also noted during continued feeding of the compound. The pine shavings used as bedding in the rat cages were stained a bright orange color for several days following administration of the compound, indicating that the acetaminofluorene or a derivative of it was being absorbed and excreted in the urine.

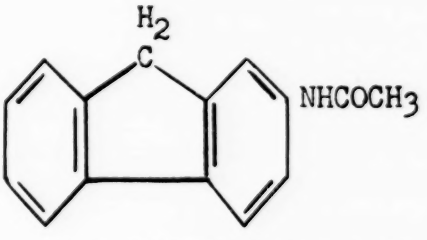


FIG. 1.—Structural formula of 2-acetaminofluorene.

* Agr. Chem. Res. Div. Contribution No. 27.

Approximately 0.5 gm. of crystalline acetaminofluorene was implanted in the subcutaneous tissue of the groin in each of 5 male rats. Neither acute reactions nor evidence of chronic poisoning, traceable to slow absorption from the depot of poorly soluble acetaminofluorene, were observed during a period of 14 months.

Each of 7 C57 mice,[1] including males and females, were injected subcutaneously with 2.5 milligrams of acetaminofluorene dissolved in sesame oil, and 42 days later the injection was repeated. No symptoms of any kind were noted during the 10 months following the first injection. Six of the mice were killed accidentally at the end of this period. The one remaining mouse was still active and in good condition 14 months after the injection.

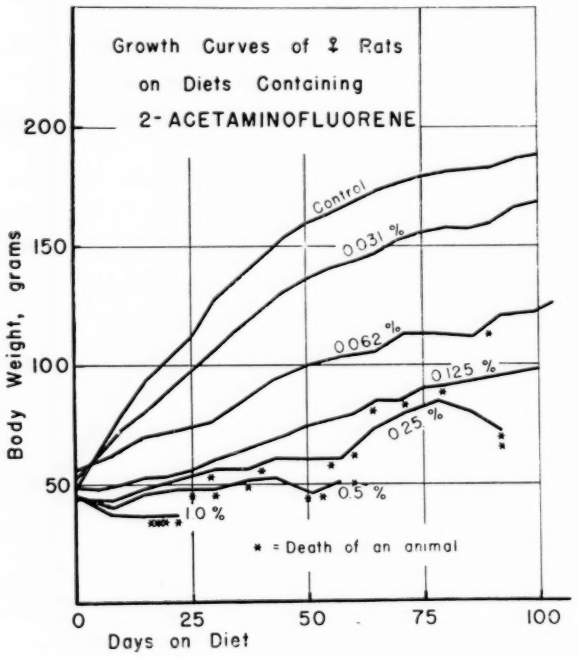
Incidental to studies on the excretion of acetaminofluorene, two rabbits were given by stomach tube 0.5 to 1.0 gm. of the compound per kg. of body weight on several occasions. One rabbit was treated twice and the other 5 times. The only change in behavior was a refusal to eat for several days following administration of the compound.

Chronic Toxicity

The continued feeding of acetaminofluorene was conducted with female albino rats weighing 45 to 55 gm. at the start. Four to 6 animals were placed in a cage. The diet to which the acetaminofluorene was added was that ordinarily used in this laboratory, and consisted of corn meal, 73 parts; linseed oil cake meal, 10 parts; alfalfa, 2 parts; casein, 10 parts; sardine oil, 3 parts; bone ash, 1.5 parts; and sodium chloride, 0.5 parts. Owing to the absence of symptoms of acute toxicity with fairly high doses, it was erroneously concluded that high concentrations of the compound could be tolerated in continuous feeding. The first trial involved dosage levels ranging from 0.125 to 1.0 per cent of the diet.

In all the continued feeding experiments which have been conducted in this laboratory, involving a variety of compounds, the results on rats adversely affected have been much the same, that is, decreased rate of growth, loss of activity, and a tendency to huddle in a corner of the cage. The animals receiving acetaminofluorene presented a marked contrast to this usual reaction. Although they failed to grow normally (Fig. 2) they were active and apparently normal in all other respects almost up to the time of death, except for a pronounced orange-yellow staining of the fur on the abdomen. Then the urethral orifice seemed to extend from the surface so that the surrounding projecting tissues had the appearance of a penis, the vagina failed to open, the animals became weak, emaciated, remained huddled, and died within a few days of the first sign of loss of activity.

It was soon apparent that all these concentrations of the compound in the diet were too high and a second



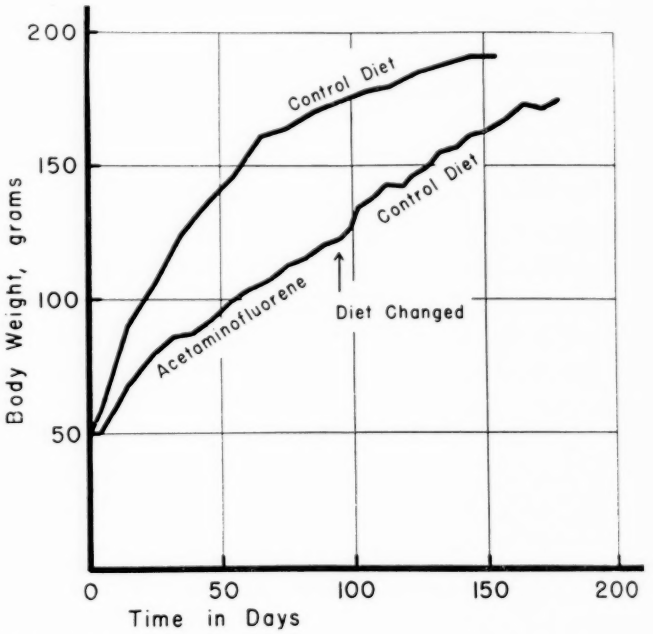


Fig. 2.—Graphs showing decreased growth and time of death of rats on diets containing 2-acetaminofluorene.

Fig. 3.—Growth curves showing lack of recovery in growth of rats after substitution of a control diet for one containing 0.125 per cent acetaminofluorene.

[1] We are indebted to Dr. John F. Menke, Finney-Howell Cancer Research Fellow in the Department of Surgery, Stanford University School of Medicine, for these mice, and for helpful suggestions during the investigation.

series of experiments was started on diets containing 0.031 to 0.125 per cent acetaminofluorene. Since with the lowest dosage, 0.031 per cent, inhibition of growth was still present as compared with the controls, a third series was started on diets containing 0.008 to 0.031 per cent of the compound. The growth curves of rats on diets containing 0.008 and 0.016 per cent of acetaminofluorene were normal and, for that reason, are not reproduced in Fig. 2.

The time elapsing between the start of the feeding experiments and the first death in a group was correlated with the concentration of the compound. All but one of the rats on the 1 per cent diet died within 22 days. This surviving rat was transferred to the control diet with a result to be mentioned later. All animals on the 0.5 per cent diet died within 60 days, and those on the 0.25 per cent diet within 93 days. Three of the 10 animals on the 0.125 per cent diet, and 1 of the 4 rats on the 0.062 per cent diet, were dead before the 100 day period was completed. It is clear that time of death, percentage mortality, and rate of growth were all functions of the concentration of acetaminofluorene in the diet.

On the 22nd day the surviving rat on the 1.0 per cent diet was transferred to the control diet. It was evident from the appearance of this animal that, if allowed to continue on the experimental regimen, it would be dead within a day or two. The rat made a spectacular recovery in appearance and rate of growth, its body weight nearly equaling that of the controls within 40 days from the return to the control diet. This result was exactly the same as that previously reported (14, 17) for rats poisoned with nicotine or cadmium and subsequently transferred to the control diet. Sixty-three days after the rat was replaced on the control diet, the experiment was discontinued.

In the studies of acute toxicity no sex difference in susceptibility to acetaminofluorene was observed. However, continued feeding of the compound did reveal such a difference. Five male rats, placed on a diet containing 0.125 per cent acetaminofluorene, were all dead before the end of 100 days, whereas only 3 of 10 females on the same dosage level died within a comparable time period. Similarly, on a dosage of 0.062 per cent, 3 of 5 males and 1 of 4 females died within 100 days. The rate of growth and the general appearance also indicated a more severe poisoning of the males. Whereas the male should weigh considerably more than the female, it was found that, on these diets, the weight of the male was no more than that of a comparably treated female. C57 mice exhibited the same sex difference, although they tolerated the continued feeding of higher concentrations of acetaminofluorene better than did albino rats.

It has been shown before with other toxic compounds fed chronically that decreased rates of growth are mainly due to a decreased food consumption, although there may be superimposed a lesser, specific effect of the poison on body weight (14, 17). It is probable that the diminished rates of growth of the acetaminofluorene-fed rats were accompanied by, or due to, a smaller amount of food eaten, but in this case it was not possible to show that this was true. The cages in this laboratory are equipped with food cups which have heretofore kept loss of food to a minimum. In spite of all precautions, wastage of large amounts of food by the rats on the acetaminofluorene diets could not be prevented. It actually appeared, from weights of food cups, as though the amount of food eaten increased progressively with the concentration of acetaminofluorene in the diet. There was another characteristic of these animals, previously mentioned, which differed markedly from that usually observed. This was the unusual activity of the treated rats. It may well be that the activity of these rats was responsible for the wasting of food. Instead of passively accepting a little of the poisoned food, the animals attempted to find some which was free of the objectionable constituent, and in so doing, spilled an excessive amount from the food jar.

Excretion

Staining of the fur of the more highly poisoned rats has already been mentioned. Moreover, the white pine shavings in the cages of the rats on the higher dosages of acetaminofluorene were stained a bright orange color, especially the shavings in the corner where the animals usually slept. Believing that the color was due to the excretion of a derivative with a quinoid structure, a group of 12 rats was placed in metabolism cages and fed on a 1.0 per cent acetaminofluorene diet for 1 week. The collected urine and washings were centrifuged to remove the food particles spilled out of the food containers. So far, we have been unable to isolate from this urine any substance with a quinoid structure. A more probable explanation of the coloration of the rat fur and pine shavings is that 2-aminofluorene, resulting from hydrolysis of 2-acetaminofluorene, is excreted in the urine. Diels (5) found that a hydrochloric acid solution of aminofluorene colored a pine stick an intense fiery red. We confirmed this observation with aminofluorene, obtained by hydrolysis of acetaminofluorene, and noted further that a more dilute solution of the aminofluorene colored pine shavings bright orange rather than red, a color indistinguishable from that on the bedding in the rat cages. We have not yet been able to isolate aminofluorene from the urine of rats or rabbits given acetaminofluorene gastrically.

Autopsy Observations

At the time of death, or after a period of 100 days on the diet, the rats were autopsied as previously described (18), the organs being weighed and saved for histological examination. All organs examined (liver, spleen, kidneys, adrenals, ovaries, and heart) were normal in size and gross appearance except the liver. Subsequently, when the production of tumors became apparent in other rats, more tissues were saved. The bladder and hypophysis were almost always removed, as well as other tissue (intestine, stomach, ureter, subcutaneous tissue) which had any indication of being enlarged or abnormal. The ovaries from the more highly poisoned rats were not as well developed as those of control animals of equal age, but corresponded closely with ovaries of animals of equal weight. The livers of rats receiving diets containing 0.031 per cent or more of acetaminofluorene were frequently nodular, and sometimes distinctly yellow. These livers weighed significantly more than those of the appropriate controls; *i.e.*, animals of the same body weight rather than of the same age (18). For example, rats on a dosage level of 0.031 per cent acetaminofluorene for 100 days had livers weighing 8.5 gm. with a probable error of 0.13 gm., as compared with 7.4 ± 0.27 gm., the liver weight of the appropriate control rats. The livers of the rats eating a diet containing 0.125 per cent of the compound for the same length of time and their controls of equal body weight weighed, respectively, 6.8 ± 0.18 and 5.3 ± 0.27 gm.

Development of Tumors

The stock colony of albino rats used in this laboratory is rather highly inbred. The animals are all descendants of 7 females and 3 males selected from our colony 3 years ago. The matings have not been brother-sister, as a rule. Our colony originated in 1931 from the Slonaker colony at Stanford University, which in turn came from the Department of Neurology of the University of Chicago nearly 40 years ago. The animals are quite uniform in rate of growth and in their reactions to the toxic agents previously studied in this laboratory. Spontaneous tumors have developed very rarely. Occasionally in the older breeding females there has appeared a large mammary tumor, but such growths have always remained localized, and histological examination of a number of them has shown benign adenoma or fibroadenoma. They may, however, have been similar to those reported by Emge (6), and possessed potentialities of malignancy. No intra-abdominal growths or tumors about the head have ever been observed. During the last few years approximately 1,500 autopsies on animals from this colony have been made carefully with histological study of all organs which were grossly abnormal, as well as many which showed no gross changes. Although several hundred of these animals were older than those used in the present study, no tumors other than the benign tumors mentioned above have been found.

Five female rats 30 days old received the 0.125 per cent acetaminofluorene diet for 95 days and were then placed on the control diet in order to learn whether they would resume a normal rate of growth after removal of the compound from the diet. As has been stated previously, replacement of the diet containing 1.0 per cent acetaminofluorene by the control diet after 22 days led to rapid recovery from the intoxication. However, this was not the case in the present experiment (Fig. 3). There was a temporary increase in rate of growth lasting about a week, after which the body weight increased at the same general rate as before. Something had happened to these rats which prevented normal recovery. On the 136th day, 41 days after the return to the control diet, one of the animals had a firm localized swelling on the side of the face just beneath the right ear. On the 144th day two more rats had developed tumors, one located in the thigh, and one in the lower abdomen. The remaining two animals had no visible external evidence of tumor formation, but when they were killed because of sickness on the 266th and 381st days, abnormal growths were present in the abdomen. These were a carcinoma of the bladder in the first instance and, in the second, an enlarged cystic liver which, microscopically, resembled a leukemic infiltration.

Several additional groups of rats, ranging from 1 to 4 months of age, were immediately placed on diets containing acetaminofluorene to confirm and extend

DESCRIPTION OF FIGURES 4 TO 8

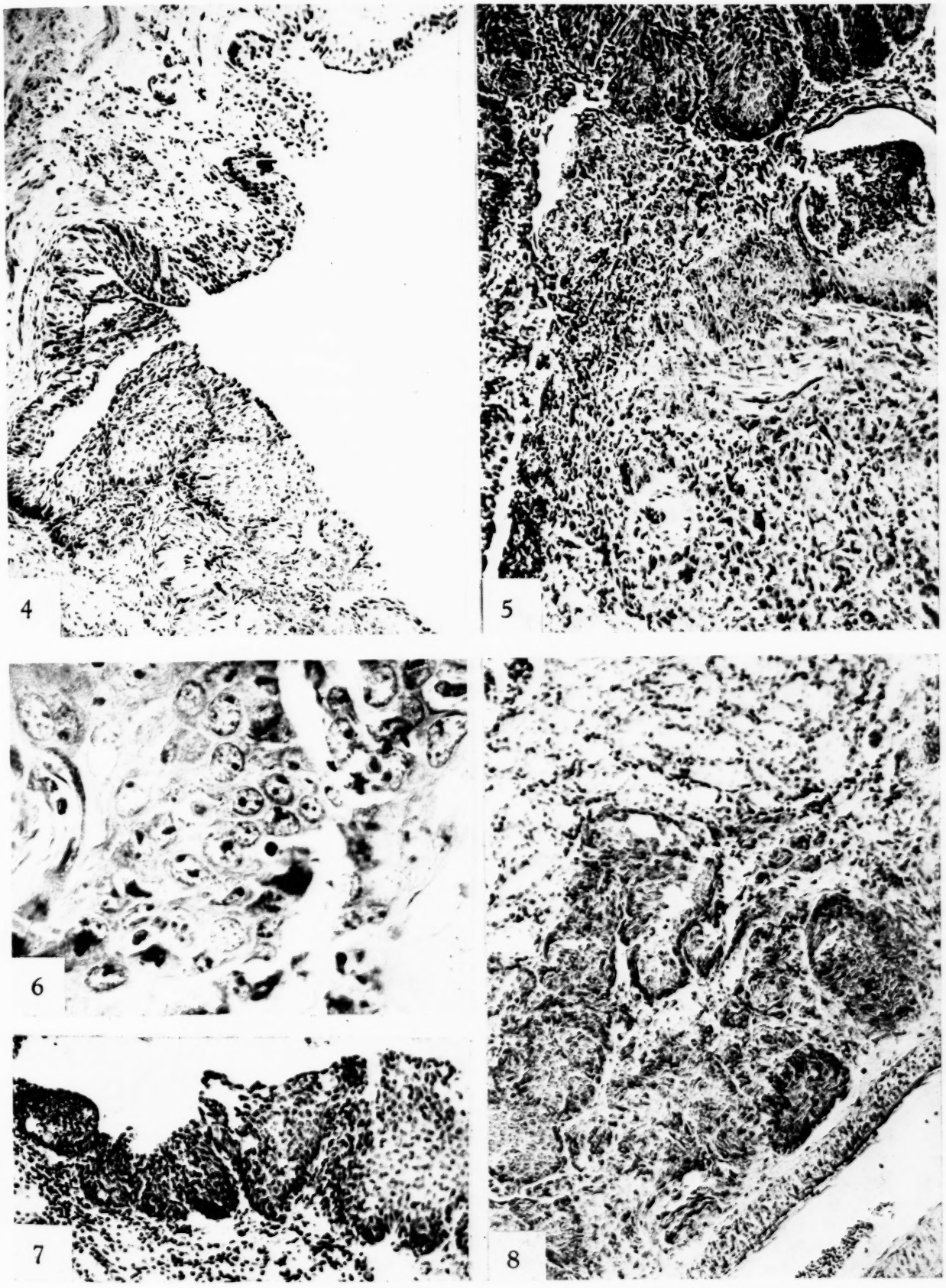
Fig. 4.—Local hyperplasia of epithelium of the urinary bladder, showing normal epithelium in the upper portion of the photomicrograph.

Fig. 5.—Carcinoma of the urinary bladder. The thickened and hyperplastic epithelium is shown at the upper edge of the photograph; the poorly differentiated invading epithelial cells at the lower right. Within one epithelial mass is a cavity filled with polymorphonuclear leucocytes.

Fig. 6.—Carcinoma of the bladder. High power photomicrograph of the deep portion of the tumor illustrated in Fig. 5, showing the edge of an epithelial pearl at the left.

Fig. 7.—Local hyperplasia of epithelium lining the renal pelvis. There is no invasion of underlying tissue.

Fig. 8.—Carcinoma of the renal pelvis invading the tissue of the kidney.



FIGS. 4 TO 8

these observations. Twenty female rats were placed on a dosage level of 0.125 per cent of the compound. Eight of these animals failed to live long enough to show any sign of tumor formation. Seven of the remainder, killed when their appearance indicated that they could live only a few days more, had histological proliferative changes in the epithelium of various organs, as will be reported below. The remaining five rats developed grossly visible tumors. These twenty rats, unlike the first group reported above, were not transferred to the control diet, and presumably were unable to live long enough to allow the corresponding development and the same incidence of tumors.

Five male rats on the same diet lived less than 100 days. Three of 5 males on half the dosage, or 0.062 per cent, were dead at this time. Of the remaining two, one showed histological epithelial changes and the other carcinomas of the bladder, liver, and pancreas when autopsied on the 199th and 213th days, respectively.

Each of 5 female rats on a diet containing 0.031 per cent acetaminofluorene developed tumors in from 251 to 280 days.

It was mentioned above that subcutaneous injections of solutions of acetaminofluorene were without an externally demonstrable effect on rats for periods ranging up to 3 months, and C57 mice were not affected in 10 to 14 months. Single gastric administration to rats of 1 gm. of the compound per kg. of body weight did not produce any signs of acute toxicity, nor have there been any tumors or symptoms of any kind during the following period of observation, now in the 14th month. That gastric administration was accompanied by absorption was shown by the excretion in the urine of a substance which stained the shavings in the cage, an excretion which persisted for several days. Approximately 0.5 gm. of crystalline acetaminofluorene was implanted in the subcutaneous tissue of the groin in each of 5 male rats. At the end of 14 months, all of the animals were in good condition, with no gross evidence of tumors. While gastric administration results in absorption and urinary excretion, as mentioned above, subcutaneous implantation or injection has behaved quite differently, as judged by the absence of colored shavings in the cages. It seems that a single gastric administration of the compound is ineffective, regardless of dose, because excretion removes it from the body before carcinogenic changes are initiated. On the other hand, the implanted crystals would presumably remain in the body for a considerable period because of insolubility. Animals receiving the implanted crystalline product a year ago have not yet been sacrificed. Even if histological changes are present in these animals, it is evident that the compound is less effective when administered by this route. The presence of colored shavings in the cages is indicative of absorption and excretion. The absence of this phenomenon, and the failure to produce tumors in the parenterally treated rats, suggest that continued administration by way of the gastrointestinal tract is necessary for carcinogenic activity.

Rough calculation indicates that rats eating the diet containing 0.031 per cent of the acetaminofluorene would ingest 300 to 350 mgm. of the compound in 100 days, or a total of about 1 gm. per kg. of body weight. This amount is comparable with the amounts mentioned above for single gastric or subcutaneous administrations. It is possible that the subcutaneously implanted crystals were gradually eliminated. As the animals have not yet been sacrificed, gross and microscopic evidence of the continued presence of the crystals is not available. As a matter of fact, due to the considerable solubility of acetaminofluorene in fats, the crystals might be dissolved in the fatty tissues and remain in the body even though not grossly observable. There is no apparent lump at the site of implantation. This suggestion of the necessity of oral administration over a period of time is tentative. The idea fits the somewhat meager data now available. Studies in progress may make it desirable to modify or discard this hypothesis.

In view of the apparent relationship of carcinogenic action of acetaminofluorene to the route of administration, it is interesting to note that "butter yellow" and certain other effective azo compounds have been administered by mouth, but these compounds are not chemically related to acetaminofluorene. Of the compounds which might be considered as bearing a chemical relationship to acetaminofluorene, beta-naphthylamine, which appears to have a carcinogenic action in dogs, seems to be the only one which has been given by mouth.

DESCRIPTION OF FIGURES 9 TO 13

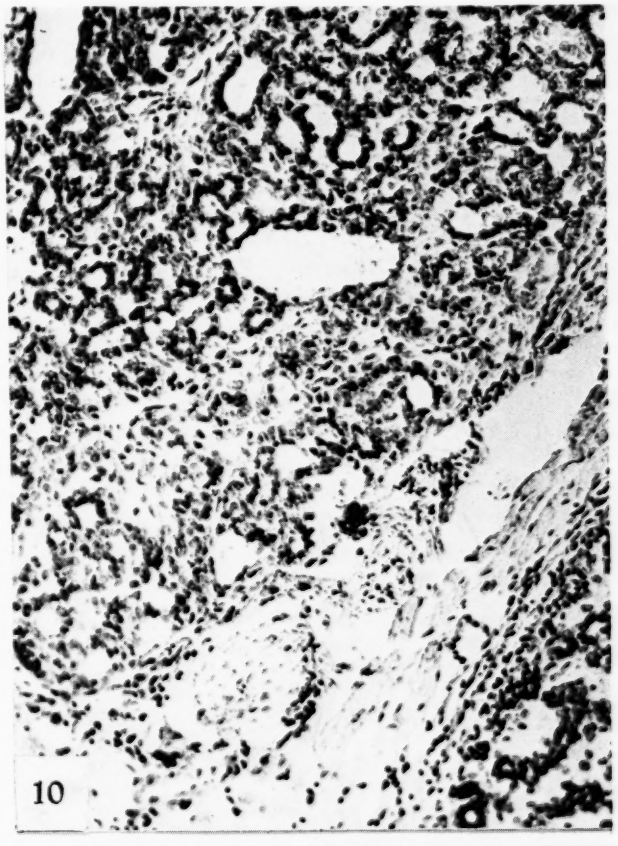
FIG. 9.—Carcinoma arising adjacent to the external auditory canal, showing several foci of beginning keratinization.

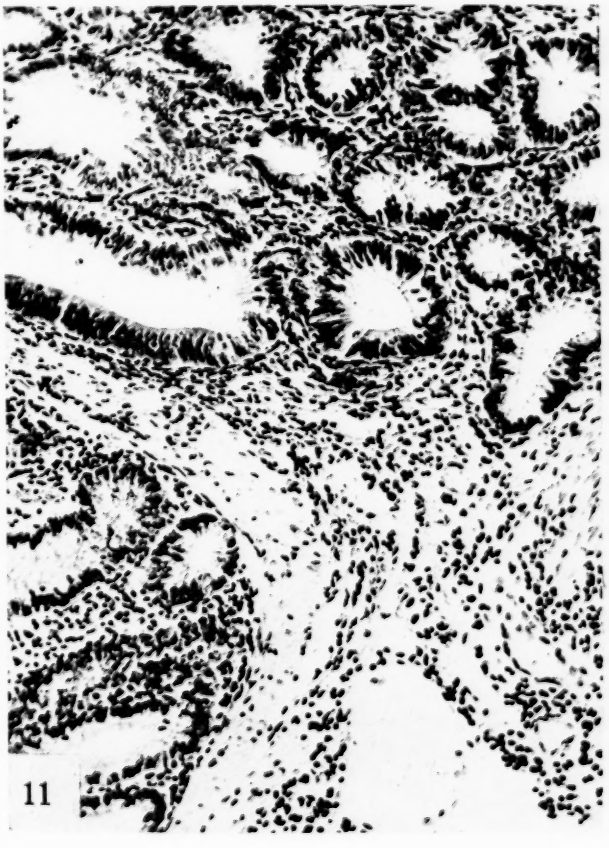
FIG. 10.—Adenocarcinoma, probably arising in the breast, invading subcutaneous tissue and skeletal muscle.

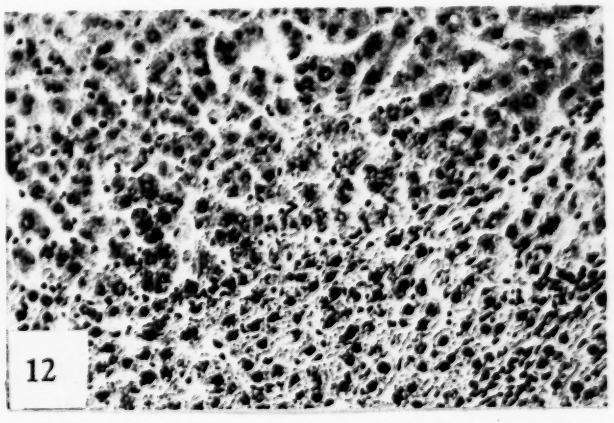
FIG. 11.—Adenocarcinoma of the colon, showing adjacent normal mucosa at the lower left and moderate lymphocytic infiltration of the tissue near the tumor.

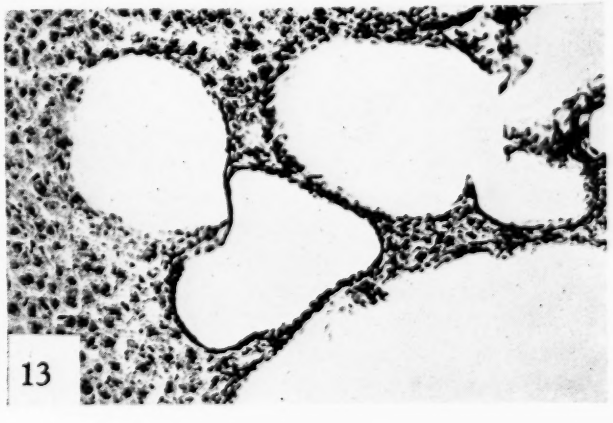
FIG. 12.—Sharply demarcated nodule of enlarged liver cells (above) bordered by normal appearing liver cells (below). There is no capsule around the nodule and no evidence of malignant growth.

FIG. 13.—A cluster of thin-walled cysts lined by simple flattened epithelium lying adjacent to normal appearing liver tissue.









FIGS. 9 TO 13

Histological Examination

Before proceeding with the report on the histological changes produced by continued administration of acetaminofluorene it is appropriate to state briefly one of the conclusions from the investigation as a whole. The duration of contact of the tissues with the acetaminofluorene or derivative in the feeding experiments is of greater import to the carcinogenic action than is the dosage. In fact, a high daily ingested dose prevents the development of neoplastic changes by killing the animal before malignancy can be initiated. Therefore, it is not surprising that tissues from rats on the higher dosages showed either no changes, or only slight hyperplastic changes and slight keratinization. The report on the histology of the tissues begins with the minor changes produced by the highest dosage level of acetaminofluorene and proceeds to the lowest, where the changes were more marked.

Paraffin sections stained with hematoxylin and eosin were used for the histological examination. Tissues from most of the animals were examined microscopically. Tissues from animals that died during the night and showed marked post mortem changes were excluded.

Most of the hyperplastic nodules observed in the different organs showed all degrees of variation from the slightest recognizable anatomical change in small groups of cells to large well demarcated nodules. It is impossible to decide at what stage such lesions should be called tumors, so they have been referred to as nodular hyperplasias, except in those instances where distinct invasion of adjacent tissue could be demonstrated. Lesions which manifested this characteristic have been regarded as malignant tumors and have been called carcinoma or sarcoma.

Three rats which had received the largest doses (1.0 and 0.5 per cent) administered for 22, 30, and 37 days, showed relatively few changes which could be attributed to the action of the acetaminofluorene. In animal No. 605, which lived 82 days after stopping administration of the compound, there were few changes of any kind. The spleen and thymus were slightly hyperplastic and there was slight irregular thickening of the epithelium of the kidney pelvis. Bladder tissue was not examined. The two rats on 0.5 per cent acetaminofluorene showed slight but definite irregular thickening of the bladder epithelium with patches of slight keratinization over the thickened areas. The livers from these animals were practically normal, although at the periphery there were a number of small collections of swollen cells demarcated sharply from adjacent liver cells, but not separated by a capsule at any point. Some of these cells contained fat droplets, while others contained no stainable fat, although they were otherwise similar. One animal (No. 600) had severe pneumonia and an abscess in the kidney which was presumably metastatic.

Of the rats which had been on the 0.25 per cent diet for 92 days, 2 were examined. They showed more pronounced changes than those receiving higher concentrations of the compound for briefer periods of time. In both animals there was marked alteration of the bladder epithelium which was thickened in places to three times the normal, and which showed marked keratinization. In many of the thickened areas there was definite metaplasia to stratified squamous epithelium. There was usually a sudden transition between the metaplastic portions and the remaining parts of the transitional epithelium lining the bladder. In the metaplastic areas there were infolding and downgrowth of thick masses of squamous cells, but the deep surface of these masses was smooth and there was no evidence of invasion of the muscle. The cells composing this atypical epithelium were large, pale staining, uniform, and occasionally showed mitosis. The kidneys were normal except for slight irregular thickening of the epithelium lining the renal pelvis, with slight but definite keratinization and accumulation of a little keratinized debris in the lumen of the kidney pelvis of rat No. 604. The livers from these animals were normal in most portions, but there were several fairly well demarcated collections of liver cells which differed distinctly from the adjacent normal cells. They were larger than normal due both to an increase in size of the nuclei and to increase in cytoplasmic volume. Some nuclei had twice the diameter of the average normal nucleus and contained large nucleoli. The cytoplasm of the large cells was less densely stained than that of adjacent cells. It frequently contained coarse granules and these were separated by spaces, suggesting that soluble material had been removed. A few of the large cells contained fatty droplets but most of them contained none. The nuclei were evenly stained and the cells in the nodules were quite uniform. The nodules often presented a sharp linear border, and in places resembled small adenomas, although at no point was there any capsule. The other organs, including the pancreas, were normal except for the lung of rat No. 603 which showed marked pneumonia.

The animals receiving 0.125 per cent acetaminofluorene showed various degrees of change, as indicated in Table I. The changes in the bladder, kidney pelvis and liver were like those described in animals which had received the 0.25 per cent diet, although they were frequently more marked, forming easily demonstrable nodular growths. In some animals invading tumors (carcinomas) of these organs had developed. In general, the greater the length of time from the start of the feeding, the more pronounced were the pathological changes. Cessation of acetaminofluorene feed-

ing after 95 days did not prevent subsequent development of tumors, as is shown clearly in Table I by the 5 animals (Nos. 772, 773, 776, 820, 836) replaced on control diet after 95 days. On the other hand, a single large administration followed by long periods on the animals. The livers of 4 animals (Nos. 609, 642, 776 and 842) contained multiple medium sized cysts up to 2 mm. in diameter lined by a single layer of flattened or cuboidal cells. These had no demonstrable relationship to the nodular masses of liver cells, al-

TABLE I: TISSUE CHANGES AFTER ACETAMINOFLUORENE FEEDING

Dose, per cent	Animal	Time on diet, days	Time until death, days	Bladder	Kidney pelvis	Liver	Pancreas	Lung	Subcutaneous tumors	Other tumors
0.125	640	103	103		+	+		−	−	
	641	103	103		+	+		−	−	
	642	103	103		−	++, c	−	−	−	
	606	104	104	−	−	+	−	−	−	
	607	104	104	Ca	+	+	−	−	−	
	608	104	104	Ca	++	+	+	−	−	
	609	104	104	Ca	+	+	−	−	−	
	772	95	150		−	+	−	−	Sarcoma	
	773	95	151		+	+		+	−	Ca (breast?)
	823	170	170	+++	−	+++	+++	−	−	
	824	170	170	+++	+	++	++	++	−	
	825	170	170	++	+	++	+++	+	−	
	826	170	170	++	−	+++	++	+++	−	
	827	170	170	++	−	+++	++	++	−	
	828	170	170	+++	+-	+-	++	+-	−	
	830	177	177	Ca					−	
	776	95	190	+	−	++, c	+	+	Ca	
	829	190	190	Ca		++	++		Ca	
	834	193	193	+	+	+			−	
	835	196	196	Ca	+++	++		++	−	Ca, ureter
	837	214	214	+	−	L	++		Ca	
	838	233	233	Ca	Ca	+++			Ca	Metastases
	820	95	266	Ca					−	
	842	273	273	++	++	+++, c	++	++		Ca, colon
	836	95	381	+		+++ L			−	
0.062	643	103	103		−	+	−	−	−	
	644	103	103		−	+	−		−	
	645	103	103		−	+	+	−	−	
	831	199	199	+++	−	+++, c		−	−	
	833	213	213	Ca	−	Ca	Ca	++	−	Metastases
0.031	646	103	103		−	+	−	−	−	
	647	103	103		−	+	−	+	−	
	648	103	103		−	−	+	−	−	
	649	103	103		+	++	−	−	−	
	650	103	103		−	+	+	−	−	
	843	302	302	++	+	Ca, c		Ca	Ca	Ca (breast?) and metastases
	844	303	303	+	++	+ c	+	−	Ca	Ca (breast?)
	845	312	312	+	+	Ca, c	+		Ca	Ad, stomach
	846	333	333	Ca	+	+, c	+	+++	Ca	

Explanation of signs:
− = no hyperplasia.
+ = slight nodular hyperplasia.
++ = moderate nodular hyperplasia.
+++ = marked nodular hyperplasia.
c = cysts.
Ca = carcinoma.
L = lesion suggesting leukemic infiltration.
AD = adenoma.

control diet did not produce tumors. Experiments are now in progress to determine the period of feeding necessary to initiate the hyperplastic changes.

The epithelium of the convoluted tubules in the kidneys frequently contained small or moderate amounts of pale brown granular pigment, the amount of which bore no relation to other changes in these though in all livers where cysts were present nodular hyperplasia was well developed. The livers of two animals (Nos. 836 and 837) were extensively infiltrated by poorly differentiated atypical cells suggesting leukemic infiltrations. Unfortunately, one of these animals was found dead and no other organs were examined. The other (No. 837) showed diffuse hyper-

plasia of the spleen with many large mononuclear cells, some in mitosis, scattered through the pulp. No other leukemic infiltrations were found. No bone marrow or lymph node tissue was available for study.

The pancreas in the majority of the animals showed frequent foci of hyperplasia of acinar cells, similar in some respects to the hyperplastic nodules in the livers. The hyperplastic pancreatic cells were often so sharply circumscribed as to have the appearance of small adenomas, although in no case were they encapsulated. The cells forming them had a distinctly different appearance from adjacent acinar cells. They were larger than normal, forming coarse, and sometimes irregular acini. As a rule they contained fewer eosinophilic granules than did adjacent cells, and in many of the more prominent nodules eosinophilic granules were absent from the cytoplasm. The nuclei were larger (up to twice normal diameter) than those of normal cells, and most of them were less deeply stained. Some, however, were hyperchromatic and an occasional mitotic figure was seen. Some cells contained multiple (up to 5) closely packed nuclei. The islet tissue was structurally normal in all animals as judged by the appearance of the hematoxylin-eosin stained material.

The bladder, kidney pelvis, liver and pancreas were the only organs among those examined in which hyperplastic changes were almost uniformly present. Also, the majority of the malignant tumors appeared in these organs, although several animals had tumors elsewhere. Four animals on the 0.125 per cent diet (Nos. 776, 829, 837, and 838) had subcutaneous epidermoid carcinomas of the side of the face, possibly arising from the auditory canal or the ducts of cutaneous glands. Rat No. 773 had an adenocarcinoma of the abdominal wall with a structure suggesting origin from breast tissue. Rat No. 835 had an early epidermoid carcinoma of the ureter. Rat No. 842 had a well differentiated adenocarcinoma of the colon. Rat No. 772 had a sarcoma of the thigh, probably arising from skeletal muscle. With the exception of the two lesions resembling leukemic infiltrations, this sarcoma was the only nonepithelial tumor which was found in any of the animals.

The lungs in the animals on the 0.125 per cent diet showed frequent changes, but it is difficult to evaluate possible effects of the abnormal diet. Of 17 animals in which the lungs were examined, 9 had well developed bronchitis and pneumonia, and in these same lungs metaplasia of bronchial epithelium and proliferation of lymphoid tissue were sometimes demonstrable. Eight animals had distinct prominence of lymphoid tissue which was made up of cells slightly larger than those of the lymphoid tissue in the normal lungs. In some cases masses of peribronchial lymphoid tissue were more than 1 mm. thick, forming nodules which were visible grossly. Metaplasia of the bronchial epithelium was seen in 5 animals. It altered the bronchial lining so that it resembled stratified squamous epithelium, although in no case was keratinization observed. In some instances metaplasia had taken place in only part of the circumference of a bronchus. Metaplastic epithelium was present only in bronchi which were surrounded by much lymphoid tissue or by pneumonic lung. In most of the animals on the diet for 170 days or longer, small structures resembling alveoli or bronchioles lined by cuboidal epithelium were numerous in this peribronchial tissue, in four cases (Nos. 824, 825, 827 and 842) suggesting adenomatous proliferation. A few mitotic figures were present in the epithelial cells, but while there was no encapsulation or sharp demarcation of these areas, no definite sign of malignancy could be found, although on a lower dosage, *i.e.,* 0.031 per cent, a carcinoma of the lung was found. The bronchi in noninflamed parts of the lungs showed no metaplasia, although in places the cytoplasm of the lining cells near the free margin was more dense and more eosinophilic than in control animals. It is impossible to state whether the bronchial changes preceded or followed the pneumonia. They may have been specific effects of the action of acetaminofluorene and they may have predisposed to the development of pneumonia. They may, however, have been sequelae of pulmonary inflammation, as inflammation was present in several animals without demonstrable epithelial changes. However, epithelial hyperplasia and metaplasia of this type have not been seen in any of the control animals studied during the last several years. Changes in other organs were not pronounced.

DESCRIPTION OF FIGURES 14 TO 18

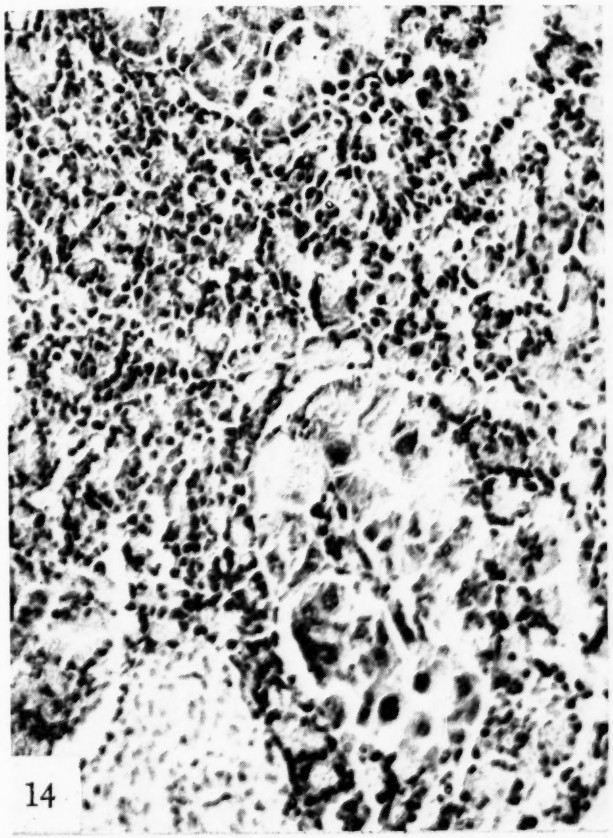
FIG. 14.—Nodular hyperplasia of the pancreas, showing a sharply demarcated but not encapsulated nodule (right center). The pale structure at the lower left is an islet of Langerhans.

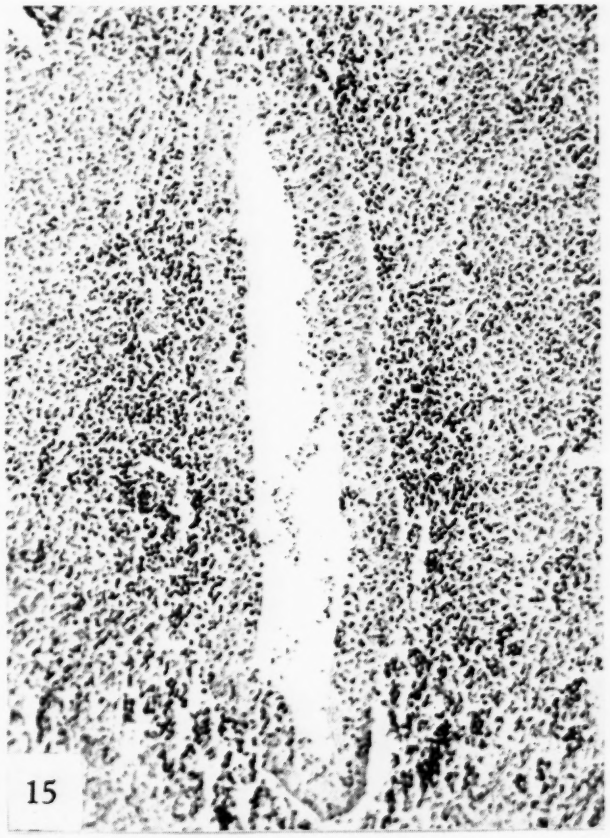
FIG. 15.—Metaplasia of bronchial epithelium, showing the lining stratified squamous epithelium surrounded by a dense mass of lymphoid tissue.

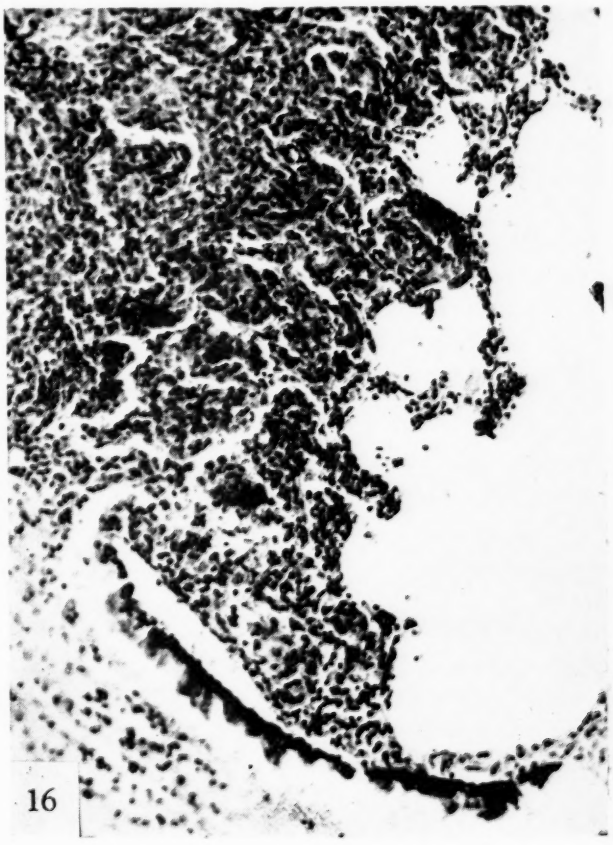
FIG. 16.—Carcinoma of the lung. Clusters of atypical epithelial cells fill the alveoli. The small bronchus contains a few inflammatory cells.

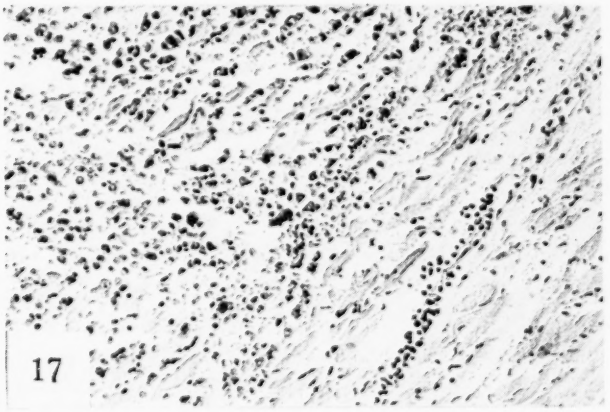
FIG. 17.—Sarcoma of the leg, showing infiltration of skeletal muscle by tumor cells.

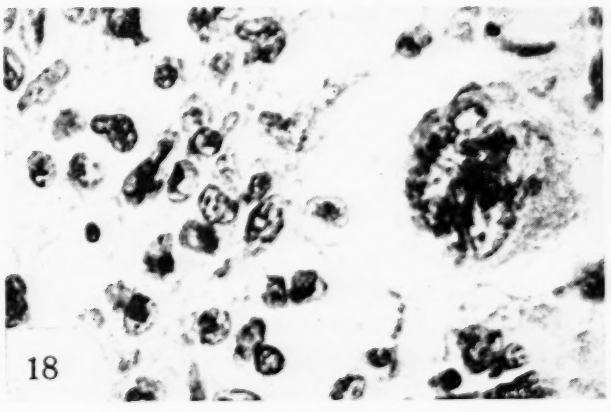
FIG. 18.—Sarcoma of leg, high power photomicrograph of tumor shown in Fig. 17. The cells vary in size and shape, nucleoli are frequently prominent, and mitoses are present. A multinucleated giant cell is shown at the right.











FIGS. 14 TO 18

The 5 animals which received 0.062 per cent and the 9 which received 0.031 per cent of acetaminofluorene in the diet showed similar changes, scarcely less pronounced than in the animals fed for the same length of time on 0.125 per cent of the compound (Table I). It was apparent in these animals, as well as those described above, that prolonged feeding (200 to 300 days) resulted in more pronounced hyperplastic changes and a greater incidence of neoplasms than occurred in animals fed the compound for only 100 days. Within the range of this series, the duration of the experiment had a much greater effect upon the character and frequency of occurrence of lesions than did the quantity of acetaminofluorene which was fed.

The lesions observed are illustrated in Figs. 4 to 18.

Discussion

The danger of predicting chronic toxicity from acute toxicity data has been clearly demonstrated by the studies on 2-acetaminofluorene. This compound which had no demonstrable acute effects on rats and mice, and affected rabbits merely by causing a temporary loss of appetite, produced marked and serious irreparable damage when fed to albino rats over a period of time. Another instance of acute toxicity differing radically from chronic toxicity is found in the case of nicotine. Here the relationship is reversed. Acute nicotine poisoning is serious, but when nicotine is mixed with the food and fed for a period of time to rats, it was shown in previous reports (14, 15, 16, 18) that all effects could be duplicated by a comparable decrease in food intake of the animals. While data on acute toxicity may help to outline a proposed investigation of chronic toxicity, it cannot be used as a substitute for that investigation.

Of 39 animals fed acetaminofluorene for 95 or more days, 19 developed tumors which were interpreted as being malignant by virtue of their invasiveness. Eight animals had multiple tumors which were thought to be unrelated to one another. There were 10 carcinomas of the bladder, 8 cutaneous or subcutaneous epidermoid carcinomas, 3 subcutaneous adenocarcinomas thought to have arisen in breast tissue, 3 primary carcinomas of the liver, 2 lesions of the liver resembling leukemic infiltrations, and 1 each of myogenic sarcoma, carcinoma of the ureter, carcinoma of the renal pelvis, carcinoma of the colon, carcinoma of the pancreas and carcinoma of the lung. Metastases from these tumors were not common, but secondary tumors were identified in 3 rats (Nos. 833, 838 and 843).

Occurring even more frequently, and evidently preceding the development of malignant tumors in many instances, was an irregular epithelial hyperplasia of the bladder, renal pelvis, liver, pancreas, and lung, in which nodules of only slightly atypical epithelial cells appeared fairly sharply demarcated, though not encapsulated, from normal tissue. Every animal showed some degree of irregular hyperplasia of at least one organ, though such changes were slight in several animals, and frequently some organs showed no recognizable hyperplasia while the change was present in other organs. No attempt has been made to distinguish between nodular areas of hyperplasia and benign adenomas, since no sharp line of distinction can be drawn.

In general, the animals which had lived the longest after the beginning of the acetaminofluorene feeding showed the most marked lesions. Differences in amount of the substance, between 0.125 and 0.031 per cent of the diet, had relatively little influence on the character and frequency of occurrence of the lesions.

The incidence of malignant tumors in the rats fed acetaminofluorene is too great to be an accidental finding. Some of the rats discussed above were killed after 100 days on the experimental diets, others after 170 days. The latter group was killed because of the poor physical condition of the animals. Of the 18 animals which, from the standpoint of other toxic symptoms, could have lived for rather considerable periods of time (because of low dosage or by return to the control diet after a suitable time of exposure to the compound), only two did not develop malignant lesions, and in one of these there was marked hyperplasia of the bladder and liver. Present results indicate that in order to produce tumors the acetaminofluorene must be given by mouth, over a period of time, and that after a suitable period the use of the compound may be discontinued without decreasing the incidence or the severity of the lesions. The present indications that the compound must be administered orally suggest that some reaction takes place after ingestion, modifying the substance in a manner not possible when it is administered parenterally. The apparent presence of 2-aminofluorene in the urine suggests that the reaction is probably a hydrolysis. Further experiments are in progress to obtain more definite information on these points. A number of closely related compounds are being studied, and observations on acetaminofluorene are being extended to include lower dosage levels and the effect on mice of the C57 strain.

Discussion of the time of first appearance of the tumors has been omitted intentionally because many of the lesions are internal and cannot be seen until autopsy. There are no external signs to indicate when malignancy begins, and there is obviously a considerable individual variation in the time of appearance of tumors in the animals studied. Three animals autopsied on the 104th day had carcinoma of the bladder. The first subcutaneous tumor was noticed on the 136th day. Death from the tumor, or autopsy due to immi-

nence of death, occurred at any time between the 150th and 381st days.

No mention of the carcinogenic properties of 2-acetaminofluorene has been found in the literature. A few compounds, more or less closely related, have been studied and will be mentioned briefly. The hydrocarbon fluorene, applied by painting on the skin or by introducing it subcutaneously, has not been found to produce tumors (3, 9, 10, 13). Fluoranthene (2, 3, 10) and chrysofluorene (2) have given the same negative results. Painting the skin with 1,2,5,6-dibenzfluorene, which resembles 1,2,5,6-dibenzanthracene except for the five-membered ring, has been found to be weakly carcinogenic (1). Hueper, Wiley and Wolfe (7) obtained papillomas and carcinomas of the bladder of dogs when they fed commercial beta-naphthylamine for 20 to 26 months. Shear (11) has produced liver tumors by the subcutaneous injection of 2-aminoanthracene. He (12) has also reported that subcutaneous injections of 9-aminophenanthrene, 3-methylamino-1,2-benzanthracene and 3-amino-1,2-benzanthracene did not produce tumors, and that tumors at the site of injection were produced by 10-amino-1,2-benzanthracene. Joseph (8) found no tumors in mice painted with 3-aminophenanthrene twice weekly for 5 months, or in which the compound had been implanted for 7 months. Most of the nitrogen-containing carcinogenic agents thus far reported have, like "butter yellow," been azo compounds.[2] In none of the reported cases have the lesions been so widespread throughout the body as was the case with acetaminofluorene.

Summary

1. 2-Acetaminofluorene was found to have no demonstrable acute toxicity for rats, in quantities up to 50 mgm. per kg., subcutaneously, and 1 gm. per kg. gastrically. One gm. per kg. by mouth to rabbits led to a temporary decrease in appetite.

2. This compound was very toxic when incorporated in the food and fed continuously to rats. No female rats lived as long as 100 days when the concentration in the food was 0.25 per cent or greater; males could not live for that length of time on concentrations of 0.062 per cent or more. Normal rate of growth was not observed unless the concentration was as low as 0.016 per cent of the diet. The more severely poisoned of the rats living for 100 days had livers which were rough, yellowish, and slightly, though definitely, heavier than normal.

3. Incorporation of acetaminofluorene in the diet in a concentration of 0.031 per cent or higher, and for a period of 95 or more days led to an irregular epithelial hyperplasia of a number of organs, especially the bladder, renal pelvis, liver, pancreas and lung. All animals showed this change to some degree, but not necessarily in all organs. In 19 of the 39 animals fed acetaminofluorene for this length of time, tumors developed which were classified as malignant by virtue of their invasive growth. In all but 3 animals, the tumors were carcinomas. There was one sarcoma and in 2 animals the lesions in the livers resembled leukemic infiltrations. The tumors were multiple in several of the animals and in 3 of these were considered metastatic.

4. Within the limits of this investigation, the time from the beginning of feeding acetaminofluorene was more important than the amount of the compound fed in producing hyperplasia and malignant growth.

[2] Since this manuscript was written, a very interesting paper by Cook, Hewett, Kennaway and Kennaway (4) has appeared. These authors found that 2,2′-azonaphthalene and the related compound 2,2′-diamino-1,1′-dinaphthyl produced changes in the livers of mice resembling those found by us in rats, while 1,1′-azonaphthalene and 1,2′-azonaphthalene and the corresponding diamino-dinaphthyl compounds did not have this action. This paper (4), combined with the reports in the literature mentioned above, and the work herein described on 2-acetaminofluorene, suggest that amino nitrogen in the 2-position possesses a biological effect differing from amino nitrogen in some other location. The wide-spread distribution of lesions in the acetaminofluorene-fed animals was not duplicated in the azonaphthalene-treated mice.

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Further Investigation on the Transmission of Induced Tumors in Fowls

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The possibility that the fowl tumor agents are of endogenous origin has been frequently suggested but no definite evidence for this idea has been produced. The demonstration of transmitting agents in induced tumors while not absolutely proof might be considered as giving support for such a conclusion. In 1925 Murphy and Landsteiner (9) reported the induction of two typical sarcomas in chickens by the injection of a high boiling point fraction of coal tar. One of these proved to be transplantable. It grew actively in new hosts, was highly invasive and metastasized freely. Repeated attempts to transmit this tumor by Berkefeld filtrates or desiccates gave negative results. In 1928 Sturm and Murphy (14) reported further tests on transmission of this tumor in which modification of methods of filtration were used, and in which the products of filtration or desiccation were brought in contact with a variety of cell types, both in adult fowls and chick embryos. Again no evidence for an agent separable from the cell could be found. Peacock (12) failed to demonstrate transmissible agents in 7 strains of transplantable induced tumors or in 8 others which were not transplantable. Mellanby (6), and Rothbard and Herman (13) among others have reported similar results. In contrast to these negative findings McIntosh (4) in 1933 reported that 3 out of his 4 transplantable induced chicken tumors were transmissible by filtrates. In 1939 with Selbie (5) he added a second series in which 2 out of 4 induced chicken tumors could be transmitted by cell-free extracts. While there has been some criticism of McIntosh's experiments, there is no immediate explanation of this divergence from the results obtained by a group of other competent investigators. The methods of induction, the system used in transplantation and filtration did not vary essentially from those in general use. The point as to whether the induced fowl tumors do contain agents separable from the cell is so important, that we consider it essential for McIntosh's observations to be confirmed by others before it is accepted. The present report is an attempt in this direction.

Transplantation

The methods of induction and description of tumors have been given in a previous paper (11). Dibenzanthracene was used as the inciting agent and this was injected either in repeated small doses in lard or in a single large dose in benzol. There were some 62 progressive tumors induced in the fowls of the group injected and from these was selected the material used for transplantation and transmission studies. All of the tumors were spindle cell sarcomas with only slight variations.

The usual procedure was to remove a piece of the tumor as soon as growth was evident; a portion was used for an autograph and the remainder for histological study. The reinoculations resulted in progressive tumors in 22 of the 23 chickens in which this procedure was carried out. These tumors or the primary ones, depending on which showed most active growth, were used for grafting into other fowls. In all, 23 of the induced tumors were used for transplantation and they were inoculated into 124 fowls. Eleven of the tumors failed to take in the first generation with a total of 40 animals inoculated. The remaining 12 tumors inoculated into 84 fowls gave positive growth in 22 (27.4 per cent). From the 12 strains in the 1st generation 6 inoculated into 19 fowls resulted in no growth. Of the remaining 6 strains inoculated into 71 chickens, 25 were positive (35.2 per cent). The 3rd generation of 66 gave 31 takes (46.9 per cent). The 4th transfer in which 48 chickens were inoculated 25 (52.1 per cent) gave progressive tumors. Some of the strains continued to give increasing numbers of takes so that by the 8th generation progressive growth took place in from 75 per cent to 100 per cent of the groups of fowls inoculated.

Transmission Experiments

The methods used for the preparation of cell-free tumor material have been Berkefeld filtrates, extracts of desiccated tumor material, and differential sedimentation of tumor extracts.

Filtration.—The tumor material freed of necrotic areas was finely minced and then thoroughly ground with sterile sand. Water in the amount of 100 cc. for each gram of tissue was added. A pH of 7.0-7.2 was maintained throughout the process by the adding of N/25 NaOH. The suspension was shaken for 20 minutes and then centrifuged at 4,000 r.p.m. to remove

the larger particles. The supernatant fluid was passed through a Berkefeld V candle and the filtrate was concentrated at 1/20 of its original volume in alundum thimbles lined with a 5 per cent collodion membrane. Kieselguhr was added to the cencentrate and 1 cc. injected into each breast of normal young Plymouth Rock fowls.

Desiccation.—Fresh tumor tissue was finely minced, spread in a relatively thin layer and placed in a vacuum jar over sulfuric acid. The chamber was evacuated and put immediately into the freezing box where it was kept till desiccation was completed.[1] The dry material was finely powdered and taken up in water in the ratio of 12 cc. to each gram of dry tissue. From 0.5 to 1 cc. of this emulsion was injected into each breast of normal young fowls.

Sedimentation.—The method used here was that developed by Claude (1, 2) for the purification and concentration of the agent of chicken tumor I. Extracts of tumor tissue were prepared as described above using 25 gm. of tissue to 250 cc. of water. The supernatant fluid after centrifugation for 20 minutes at 4,000 r.p.m. was strained through sterile cheese cloth to remove the fatty material which floats on the surface. A pH of 7.0-7.2 was maintained throughout the procedure. The solution was then spun at 18,000 r.p.m. for 1½ hours. The sediment from all the tubes was collected and resuspended in 8 cc. of distilled water. The pH was again adjusted to 7.0 and this extract sedimented at 18,000 r.p.m. for 4 minutes. The supernatant was put aside and the sediment resuspended in 8 cc. of water and again spun for 4 minutes. This process was repeated twice more, giving 4 spinnings of 4 minutes each which resulted in the removal of the larger particles with a minimum loss of the smaller ones. The supernatant fluids from these four centrifugations were pooled and the total amount subjected to 1½ hours of centrifugation at 18,000 r.p.m. The sediment from this was resuspended in 8 cc. of distilled water. This formed the material for tests and 0.5 cc. was injected into each breast of normal young chickens.

The only variation in the above procedure in the different tests was that in some the extracts were prepared from tumor tissue freshly removed from the animal while in others, the tissue was kept from 16 hours to 6 days in a frozen state. The reason for this is that chicken tumor I kept frozen for a time gives a larger yield of the transmitted agent than when fresh tissue is used.

[1] It was demonstrated by Murphy in 1911 that the chicken tumor I agent withstood drying. Later he demonstrated that if the tumor material was kept frozen during the desiccation process, the activity of the agent was little impaired and could be kept over long periods. In 1929, Hawkins in this laboratory reported that several viruses, dried in the frozen state, showed no appreciable loss in virulence after a year in storage. (Hawkins, J. A., Proc. Soc. Exper. Biol. & Med., **26**:479-480. 1929.)

With any of the above methods the agent of chicken tumor I may be secured in highly active form. The most satisfactory of these methods in transmitting this tumor is by sediments. This material diluted up to the volume of the original extract has a greater tumor producing activity than the full unfiltered extract from which it is prepared. This increase in activity is undoubtedly due to the fact that the method of preparation eliminates the inhibitors which are known to be present in crude extracts and filtrates (10). The concentration of this factor may be so great as to neutralize the tumor agents. This was true in one chicken tumor which was not transmissible by filtrates or desiccates prepared in the usual way but when the inhibitor was eliminated by sedimentation, transmission was easily accomplished (3).

A total of 36 tests was made on different generations of 8 strains of transplantable induced tumors. In all 150 chickens were inoculated with the cell-free products divided as follows: 118 chickens with the sedimented material, 18 with concentrated Berkefeld filtrates, and 14 with the suspension of desiccated tumors. In the grand total of 150 each having two sites of inoculation, totaling 300 tests, not even a single suspicious tumor arose. The details of the tests are given in Table I.

In addition to the above experiments, sediments from two actively growing tumors of strain 870 were tested on the chorio-allantoic membranes of chick embryos. This has been shown by one of us to be a delicate indicator for the agents of the filterable tumors.

Cells from these induced strains grow readily when placed on the chicken membranes but in three tests involving 30 embryos, neither the extract nor the sedimented fraction caused tumor formation.

Effect of X-ray on Induced Tumors [2]

It has been established that the transmitting agents of the filterable chicken tumors are remarkably resistant to x-ray, while the tumor cells can be easily destroyed by irradiation. The following experiments were carried out in the Department of Pathology, Cornell University Medical College, by Messrs. H. C. Miles, R. G. Marquardt, and E. H. Tuttle under the Staff of the Leukemia Funds.

The tumors to be investigated were cut up into fine particles and half of these were irradiated by x-rays using the following factors: 140 kv., 5 ma., 9 cm. distance, and no filter. This combination gave 887 r per minute, with 23 minutes required to deliver 20,000 r units to the tumor particles. During this time the material was kept on an iced pan. The chicks for the tests varied in age from 1 to 4 weeks, but in each

[2] Through the kindness of Dr. Jacob Furth we are able to include these interesting results in the present publication.

experiment those used were of the same age. As previous experiments have shown that x-ray increases susceptibility to inoculated tumors, all of the chicks were irradiated with 250 to 400 r units before inoculation. Each one was injected in both breasts and legs. two strains 11 and 13 of spontaneous origin from the series of Drs. Furth and Stubbs.

This extensive material gives still further evidence of the absence of transmitting agents in the induced chicken tumors. The amount of x-ray given is shown

TABLE I: TRANSMISSION EXPERIMENTS*

Tumor strain number	Generation	Age of tumor	Number injected	Condition tumor tissue	Method
474	2nd	6 wks.	5	Frozen 16 hrs.	Sedimentation
	3rd	8 wks.	3	Fresh	Sedimentation
475	2nd	10 wks.	5	Fresh	Sedimentation
	3rd	10 wks.	3	Frozen 18 hrs.	Sedimentation
491	2nd	10 wks.	4	Frozen 48 hrs.	Sedimentation
753	2nd	4 wks.	4	Frozen 16 days	Sedimentation
	6th	16 days	5	Fresh	Sedimentation
	7th	3 wks.	5	Frozen 18 hrs.	Sedimentation
	8th	3 wks.	5	Frozen 16 hrs.	Sedimentation
	10th	3 wks.	5	Fresh	Sedimentation
808	5th	16 days	5	Fresh	Sedimentation
	8th	3 wks.	3	Frozen 24 hrs.	Sedimentation
281	4th	4 wks.	5	Frozen 48 hrs.	Sedimentation
	6th	3 wks.	5	Frozen 24 hrs.	Sedimentation
	8th	3 wks.	5	Frozen 48 hrs.	Sedimentation
	9th	4 wks.	5	Fresh	Sedimentation
	9th	3 wks.	5	Frozen 48 hrs.	Sedimentation
	10th	3 wks.	2	Fresh	Sedimentation
870	5th	4 wks.	5	Fresh	Sedimentation
	5th	4 wks.	5	Fresh	Sedimentation
	6th	3 wks.	5	Fresh	Sedimentation
	7th	3 wks.	3	Frozen 5 days	Sedimentation
	8th	3 wks.	3	Frozen 4 days	Sedimentation
	9th	3 wks.	4	Fresh	Sedimentation
	10th	3 wks.	4	Frozen 6 days	Sedimentation
	12th	3 wks.	5	Frozen 3 days	Sedimentation
287	2nd	4 wks.	5	Frozen 3 days	Sedimentation
753	8th	3 wks.	5	Frozen 16 hrs.	Conc. Berk. filt.
281	9th	4 wks.	4	Fresh	Conc. Berk. filt.
	10th	3 wks.	2	Fresh	Conc. Berk. filt.
870	7th	3 wks.	4	Fresh	Conc. Berk. filt.
808	8th	3 wks.	3	Frozen 1 day	Conc. Berk. filt.
870	12th	3 wks.	4	Desiccated	Emulsion of desiccate
474	2nd	6 wks.	4	Desiccated	Emulsion of desiccate
	2nd	4 wks.	2	Desiccated	Emulsion of desiccate
475	3rd	10 wks.	4	Desiccated	Emulsion of desiccate

* Total—8 strains; 150 chickens inoculated: 118 with sediment; 18 with Berkefeld filtrates; 14 with desiccate.

The details of the experiments with the results are given in Table II.

The following tumors were used in the tests: Strain 16, originally induced by injections of methylcholanthrene and transplanted in Dr. Furth's laboratory; strain 870 induced by dibenzanthracene in our laboratory and is included in the group reported above; and to be without perceptible effect on the agents of the two filterable tumors, included in the experiments. From other studies it is known that the filterable agents of tumors will withstand far greater doses of x-ray without damage to their tumor producing activity. If transmitting agents existed in the induced tumors it would be expected that some evidence of

TABLE II: EFFECT OF X-RAY ON TRANSPLANTS OF INDUCED TUMORS

Tumor used	Untreated tumor tissue			X-rayed tumor tissue				Cell-free filtrate		
	No. inoc.	No. positive	Per cent	Dose	No. inoc.	No. positive	Per cent	No. inoc.	No. positive	Per cent
Strain 16										
Exp. 1	8	8	100.0	10,000 r	20	11	55.5	24	0	0.0
Exp. 2	24	20	83.3	10,000 r	24	0	0.0			
Exp. 3	24	20	83.3	20,000 r	16	0	0.0			
Exp. 4	16	11	68.8	20,000 r	28	0	0.0	32	0	0.0
Exp. 5	24	17	70.8	20,000 r	32	0	0.0			
Exp. 6	20	12	60.0	20,000 r	20	1	5.0	40	0	0.0
Strain R. I. 870										
Exp. 1	16	16	100.0	20,000 r	20	0	0.0			
Exp. 2	20	20	100.0	20,000 r	24	0	0.0	40	0	0.0
Controls										
Sarc. 11	24	24	100.0	20,000 r	24	24	100.0			
Sarc. 13	24	24	100.0	20,000 r	24	24	100.0			

their presence would manifest itself after this comparatively mild treatment which is barely sufficient to inhibit the growth of the tumor cells. It is of interest to note that strain 870 gives quite regularly 100 per cent takes and is more rapid in its growth than many of the transplanted tumors of spontaneous origin which are easily transmitted by filtrates.

DISCUSSION

The importance of the question as to whether induced fowl tumors contain a filterable agent justifies a continued investigation. As noted above numerous tests by several workers have failed to demonstrate transmission of induced fowl tumors except by living tumor cells. On the other hand McIntosh (4, 5) has reported 5 induced tumors which he claims are transmissible by filtrates. It was difficult to estimate the first of McIntosh's results, for leukemia appeared in his strains and at least one of the tumors arose at a distance from the tar injection. These complications do not appear to come into his second series. In this present report there is added another series of transplanted induced tumors, some 8 strains, in which repeated attempts to transmit by cell-free materials have given only negative results.

The methods used by McIntosh seem to vary in no essential particular either in the manner of induction or method of filtration from those used by other investigators. Nor do the tumors he studied appear to have been of a higher grade of malignancy or to have been more easily transplanted.

A definite answer in either direction would open up some interesting grounds for speculations. If we could accept the results of the majority of tests as the correct answer and accept the induced tumors as true neoplasms, it might throw some doubt on the classification of the filterable fowl tumors which still occupy a unique place in the tumor group. Could it be that cells may become malignant in two ways as has been suggested by one of us (7, 8) *i.e.* through a mutation, to have lost a controlling factor or to have acquired the ability to produce an excess of a growth factor? Why should there be this sharp difference between the induced and spontaneous fowl tumors? All of the tumors of the latter class, if properly investigated, have given evidence of containing a transmissible agent. Failure in at least one strain, a very slow growing tumor, was due to the presence of a strong inhibiting factor and when this was eliminated the active agent was found in abundance. The methods used in the present experiments eliminate the inhibitor so that the failure in transmission can not be attributed to this factor. It is unfortunate for the better understanding of the chicken tumor group that McIntosh's observation can not be confirmed in other laboratories. It is our opinion that it is necessary to reserve the acceptance of his results in as much as at least three groups of competent workers with a large number of tests have failed to confirm them.

SUMMARY

Tests on 8 strains of chicken tumors induced by carcinogenic chemicals have failed to give any evidence of transmissible agents separable from the tumor cells. The methods used for preparing the material included desiccation, filtration, and high speed sedimentation, all of which methods have proved successful in securing the active agents from fowl tumor strains of spontaneous origin. The cell-free products of the induced tumors were tested by 300 inoculations in 150 young fowls of the same breed as that in which the tumors were induced. A report is also included which shows that x-ray given in sufficient amount to damage the tumor cells, but insufficient to inactivate the filterable agents, destroys the transplantability of the induced tumors. This more extensive study confirms results of our earlier experiments and those of others, showing that the induced fowl tumors like the tumors in mammals can be transmitted only by intact living tumor cells.

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The Extraction of a Carcinogenic Fraction from Human Urine*†

(Preliminary Report)

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The structural characteristics of synthetic carcinogenic substances, estrogens, and androgens have suggested to many the possibility that an abnormal metabolism of sterols or a disturbed elimination or destruction of carcinogenic steroid intermediates might be involved in human cancer. The fact that human urine contains relatively large amounts of conjugated androgens and estrogens in water-soluble forms led us to investigate human urine as a possible source of carcinogenic substances, possibly steroid in type. Accordingly, a systematic extraction of human urine was undertaken and the crude fractions obtained were tested for carcinogenic activity in mice.

Studies by others on various urinary preparations have not led to definite conclusions. Bischoff and Maxwell (1) reported that a "Kallikrein" or depressor fraction, which is difficultly soluble in 50 to 80 per cent ethanol may augment the number of takes after tumor inoculation in rats. Turner (6) and Bowman and Mottshaw (2) studied the effects of the very crude alcohol-soluble growth-stimulating and the difficultly alcohol-soluble growth-inhibiting fractions (4) from urine on tumor growth. The former (6) concluded that the growth-stimulating fraction from normal urine has no effect on the rate or type of tumor produced by carcinogenic agents. He indicated a slight inhibiting effect from the growth-inhibiting fraction. Bowman and Mottshaw (2) reported that neither of the fractions above, nor ether, nor benzene extracts from the urine of cancer patients produced tumors in mice in 250 days. Brikker and Timofejewa (3) and Sobotka and Bloch (5) reported negative results with benzene and butyl ether extracts from the urine of cancer patients in tests observed for 5 months. Sobotka and Bloch (5) also reported a 50 to 75 per cent recovery of 100 milligrams phenanthrene and dibenzanthracene added to 20 liters of urine.

Sarcomas Developed at the Site of Injection in Mice Injected with an Extract of Human Urine

The percentage yield of the tumors was low so that repetition and extension of the work is necessary and is under way. Pending the conclusion of these new experiments, which because of the long induction time (minimum of 17 months) will be about 2 years, we are making this preliminary report. Also it is important that others who might be working with similar urine extracts appreciate this long induction time so as not to end their experiments prematurely.

Preparation of Urine Extracts [1]

All the urine was from men. The normal urine was collected daily from urinals. The cancer urine was from patients suffering from cancer not involving the endocrine system as far as could be determined. As this was necessarily a preliminary study all the extracts were very crude mixtures. A brief description of the various types of extracts follows:

All the extractions were conducted under diminished pressure in a modification of the extractor used by the Department of Biochemistry, University of Chicago, for the quantitative extraction of sex hormones from urine.

Benzene extract without hydrolysis.—The urine at pH 3.4 to 3.8 was extracted at room temperature with benzene until at least 12 volumes of benzene had passed through the urine. In order to diminish the foaming, large amounts of sodium chloride were added to the urine. This extract should contain all the free androgens, estrogens, other steroids, phenols, fatty acids, fats, some pigments, etc. By shaking with saturated aqueous sodium bicarbonate, acids and much pigment were removed.

Butyl alcohol extract.—The urine after this treatment was extracted with normal butyl alcohol in the same apparatus. This extract should contain conjugated forms of steroids, estrogens, and androgens, and some water-soluble nitrogenous extractives.

Benzene extract after acid hydrolysis.—The urine after the butyl alcohol extraction was acidified by HCl to pH 1.0, boiled for 15 minutes, cooled, and re-extracted with benzene in the same way.

* This investigation was aided by a grant from the National Advisory Cancer Council.

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[1] We are indebted to Dr. Gerald R. Allaben of the Edward Hines, Jr. Hospital, Broadview, Illinois, who arranged for the collection of some of the urine from patients with cancer.

Neutral fraction of the first benzene extract.—The first benzene extract was very toxic, hence it was fractionated by shaking an ether solution of the solids three times with saturated aqueous sodium bicarbonate. The ether solution left is referred to as the "neutral fraction."

50 per cent alcohol-insoluble fraction from the first benzene extract.—The ether solution above was evaporated to dryness, dissolved in ethanol, and then diluted with an equal volume of distilled water. The tarry precipitate which separated is the fraction referred to here.

Petroleum ether-soluble fraction from the 50 per cent alcohol-soluble fraction.—This was obtained by extracting the 50 per cent alcoholic solution above with petroleum ether.

Table I: Lack of Carcinogenicity of Various Fractions of the First Benzene Extract of Human Normal and Cancer Urine *

Description of urine extract	Sex of mice	Survival time in months							
		0	6	9	12	15	18	21	24
Neutral fraction of the first benzene extract—normal.	Female	11	11	10	8	6	5	5	2
Neutral fraction of the first benzene extract—cancer.	Female 2 Male 5	7	6	6	4	3	3	2	1
Petroleum ether-soluble fraction from the 50 per cent alcohol-soluble fraction of the first benzene extract—normal.	Female	5	5	5	5	4	3	3	3
50 per cent alcohol-insoluble fraction of the first benzene extract—normal.	Female	7	5	4	3	3	1	0	
50 per cent alcohol-insoluble fraction of the first benzene extract—cancer.	Male 2 Female 6	8	8	8	6	5	5	3	

* The doses per mouse represent the equivalent of 5 to 15 liters of urine.

Method of Testing the Extracts for Cancer-Producing Ability

The urine extracts were dissolved in sesame oil. Some of them remained in solution poorly and it was necessary to warm them to the boiling point of water to get them into a syringe for injection. Each mouse was injected once subcutaneously in the interscapular region with the desired amount of urine extract contained usually in 0.5 cc. of sesame oil. The mice used were of our own albino stock. They were about 8 weeks old, and of the sex indicated in Tables I to III. Spontaneous sarcomas, other than lymphosarcomas, have never been seen in this partly inbred colony in several thousand individuals over a period of nearly 3 years, although mammary gland tumors, lung tumors, and leukemic diseases are spontaneous in them.

Experiment A. Carcinogenicity of various fractions of the benzene extract of urine (without hydrolysis).—Various fractions of human urine, normal and cancer, as indicated in Table I, were injected in one dose of 100 mgm. per mouse. The sex of the mice and the survival time in months up to the end of the second year are shown. No sarcomas occurred at the site of injection in these experiments. Despite the negative results these experiments are included in this report in order that unplanned duplication of effort may be avoided by others, and because they serve as control experiments for our stock of mice and for our specimen of sesame oil.

Experiment B. Carcinogenicity of the butyl alcohol urine extracts.—This extract was injected in doses of 100 mgm. per mouse given once. Seventeen male mice were injected with the extract from cancer urine, and 17 females were injected with the noncancer urine extract. The mice were from 37 to 59 days old.

The urine extracts had a slight primary toxicity, one animal in each group of 17 dying within 24 hours. The delayed caustic effects of the injected extracts were slight, only an occasional small slough being noted. Soft, fluctuant masses persisted at the site of injection of some mice for many months after injection. The unfortunately high mortality in the mice injected with the cancer urine extracts was due mostly to renal infection, the result of fighting wounds around the genitalia in these males. The results of these experiments are summarized in Table II.

Table II: Tumor Production in Mice by Butyl Alcohol Extracts of Human Urine *

Time in months	Urine from cancer persons		Urine from noncancer persons	
	Number mice living	Number mice dead with sarcoma	Number mice living	Number mice dead with sarcoma
0	17	0	17	0
9	16	0	16	0
12	5	0	16	0
17	1	1	14	0
20	0	1	14	0
21	..	..	11	1
23	..	..	9	2
24	..	..	8	2
26	..	..	6	2

* The dose per mouse was the equivalent of 80 to 125 cc. urine.

Three tumors have occurred at the site of injection and 6 mice are still alive and without visible tumors at 26 months. One of these tumors was present in a mouse which had been injected with cancer urine extract, dying in the 17th month. At this time only one other mouse was alive in this group and he died subsequently without tumor. Two tumors have appeared in the mice injected with noncancer urine extracts. The first of these died with tumor in the 21st month, at which time 11 mice were alive in the experiment, and the second died in the 23rd month. Considering the induction time as 17 months, the effective total tested was 16 mice, and the percentage yield was 18.7 per cent (3 tumors in 16 mice).

These 3 tumors are fibrosarcomas in which the cells are pleomorphic and the collagen abundant (Fig. 1). Grossly they were first recognized as tiny, hard masses immediately beneath the skin to which they were adherent. They showed early ulceration. They grew slowly and spread laterally beneath the skin to form flat, fixed, infiltrating tumors which measured $12 \times 10 \times 6$ mm.; $24 \times 22 \times 16$ mm.; and $25 \times 21 \times 10$ mm. at the time of death. One metastasized to the lungs. Because of the ulceration with infection attempts at transplantation were not made.

Tumors also occurred in the lungs, the mammary glands, and the lymphatic system of some mice in this experiment. They are not counted as induced tumors because at the present time it is not certain that their number exceeded the expectancy of spontaneous tumors for this stock of mice.

The three sarcomas which occurred at the site of injection of the urine extracts are different in several respects from the sarcomas which are usually induced by carcinogenic hydrocarbons. They were different in their long induction time, slow rate of growth, flat infiltrating type of tumor with early ulceration, and by their scirrhous, pleomorphic microscopical appearance. Whether this behavior is due to low dosage or low potency of carcinogen, or to other factors we cannot say. It is not attributable to any peculiarity inherent to the mice used, as is indicated by the following experiment.

Experiment C. Tests of the butyl alcohol extracts from human urine for factors which modify tumor production by methylcholanthrene.—Male mice of the same stock as those used in the previous experiments were each injected once with 100 mgm. of the butyl alcohol-urine extracts and to which 1 mgm. of methylcholanthrene was added. Another group of mice was injected with methylcholanthrene alone. This experiment was planned to see if there might be either acceleration or retardation of tumor production by the methylcholanthrene when the urine extract was added to it.

The results are given in Table III. The tumors listed there are all spindle or mixed cell sarcomas occurring at the site of injection. There is no important difference in the induction time or the percentage yield of tumors in these three groups of mice, or in the gross and microscopical features of these tumors, which resemble those found in other experiments in which the chemical carcinogens are used. Most of the deaths in nontumor-bearing mice were due to fighting wounds and their sequelæ.

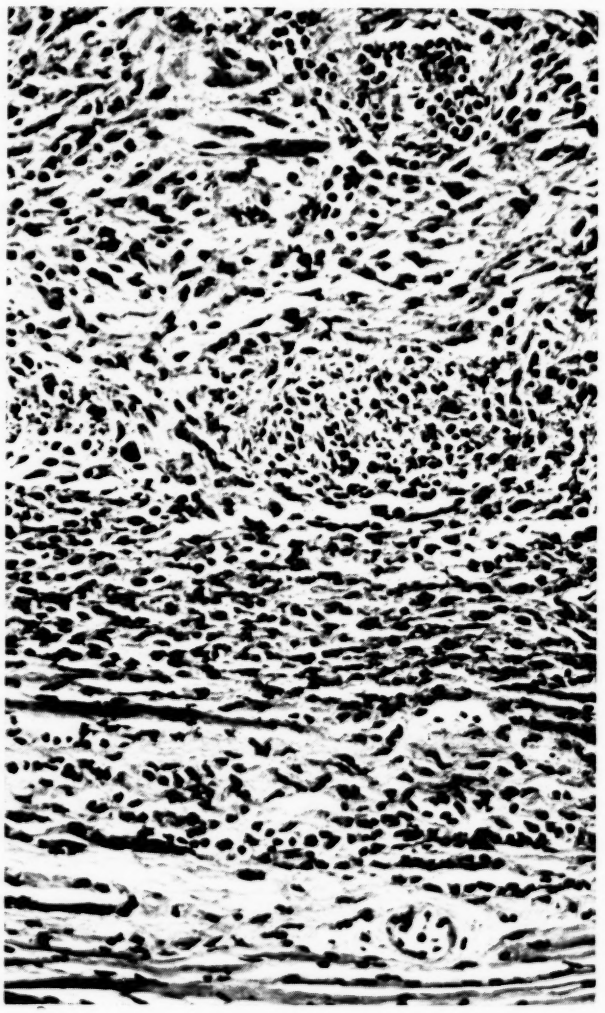

FIG. 1.—Photomicrograph of section of pleomorphic cell fibrosarcoma infiltrating muscle in a mouse injected subcutaneously with butyl alcohol extract of urine from noncancerous persons. Mouse died 21 months after injection. Mag. ×285.

This experiment shows that tumor induction by methylcholanthrene was not inhibited or accelerated by a co-injection of an extract of human urine from cancer and noncancer persons, in the dosages used. It further shows that this stock of mice is reasonably susceptible to tumor induction by methylcholanthrene (a high percentage of those alive when the first tumors

occurred developing tumors), and that the explanation for the long induction time, the low percentage yield of tumors, and the peculiar morphology of the three tumors reported in experiment B does not lie in the stock of mice used.

TABLE III: EFFECT OF BUTYL ALCOHOL EXTRACTS FROM HUMAN URINE ON TUMOR PRODUCTION BY METHYLCHOLANTHRENE IN MICE

	Cancer urine extract with methylcholanthrene *		Noncancer urine extract with methylcholanthrene *		Methylcholanthrene alone	
Time in months	Number of mice living	Dead with tumor	Number of mice living	Dead with tumor	Number of mice living	Dead with tumor
0	16	..	15	..	18	..
1	14	..	11	..	18	..
2	14	..	10	0	16	..
3	14	0	9	1	14	0
4	11	3	9	1	8	1
5	5	8	4	6	4	6
6	5	8	3	6	1	9
7	5	8	2	7	1	9
8	5	8	2	7	1	9
9	4	8	1	8	1	9
10	4	8	1	8	1	9
12	3	9	1	8	1	9
16	0	9	0	8	0	9

* Each mouse received one injection of the equivalent of 80 to 125 cc. urine.

DISCUSSION

Special comment will be made on the possible nature of the material found in the butyl alcohol extract. In view of the preliminary exhaustive extraction by benzene we would expect the butyl alcohol extract to be relatively free from free androgens and estrogens. Hence the carcinogenic action, if it is confirmed later, might be due to conjugated forms of one or both of these groups, to other steroid combinations having these peculiar solubilities, or to nonsteroid water- and butyl alcohol-soluble extractives of varying chemical character. It would be very remarkable if the water-soluble conjugated estrogens and androgens from 80 to 100 cc. of human urine were found responsible for these tumors, first because of the water solubility and second because of the low dosage involved. Hence the indications are that the carcinogenic action probably is due to other constituents of which there are many kinds in this crude extract. The fact that only 100 mgm. of this crude material showed some carcinogenic activity encourages us to attempt the confirmation of these observations and the identification of the carcinogen.

SUMMARY

1. Benzene extracts from unhydrolyzed human normal or cancer urine, whether injected as such or after further fractionation, did not produce tumors in albino mice.

2. The butyl alcohol extracts from such urines previously extracted with benzene produced 3 fibrosarcomas in 16 to 23 months after one injection into 34 mice. The effective total was 16 mice, and the percentage yield of tumors was 18.7. One tumor occurred after the injection with extract of cancer urine and 2 after injection of extract of normal urine.

3. Such butyl alcohol extracts, when combined with methylcholanthrene, did not retard or accelerate the tumor induction in white mice. The tumors produced by methylcholanthrene alone or with butyl alcohol extracts of urine were morphologically identical, but differed from those produced by the urine extract alone.

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The Production of Tumors by Transplantation of Normally Appearing Liver Cells from Animals Previously Injected with Methylcholanthrene*†

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Several years ago we observed that injected minced liver of C3H mice, previously given methylcholanthrene and bearing subcutaneous sarcomas of the groin, occasionally resulted in rapidly growing tumors at the site of injection. As such tumors usually developed within three weeks and resembled the primary methylcholanthrene growth (polymorphous cell fibrosarcoma), it seemed probable that living cells of a metastasis had been transferred.

Repeated histological studies of samples of liver used in transplantation, however, failed to reveal metastases in the liver. In fact, little or no abnormality was found in livers giving successful takes. Such tissues showed suspicious cells only occasionally. Variations in nuclear size, hyperplasia and mitoses (the mitoses being in liver cord cells rather than in secondary tumor cells) were observed. A few liver cells contained suggestive intranuclear inclusion bodies. Occasional multinucleated cells, possibly megakaryocytes, were seen not only in the liver sinusoids but also in the spleen. These cellular features, however, could be found in normal animals of the same age. The significant point is that although tumor metastases were not visible, either grossly or microscopically, rapidly growing tumors were occasionally found to develop at the site of injection of such seemingly normal liver tissue.

Stimulated by the recent publications of Steiner (4), Kleinenberg, Neufach, and Shabad (3), Des Ligneris (1), and Hieger (2), who reported the production of tumors in mice by the injection of lipoid extracts of human liver, we extended the original transplantation experiments. These are reported here.

Experiments

The material for this study was derived from: I. 62 C3H mice bearing sarcomas induced by methylcholanthrene, measuring 1 to 2 cm. in diameter; II. 21 C57 black mice bearing methylcholanthrene tumors of similar size; III. 30 C3H mice injected 7 weeks previously with methylcholanthrene but not yet possessing induced tumors; and IV. 37 normal C3H mice.

The freshly excised sterile liver of each host was cut into 6 or 8 pieces, several of which were selected at random for fixation in formalin for microscopic study. The remaining pieces were minced and to the hash an equal volume of saline was added. Approximately 0.2 cc. of this minced tissue was injected subcutaneously into the groin area of 5 to 10 recipient mice. In more recent experiments a concentrated cell-free filtrate,[1] obtained from thoroughly ground liver, was injected into 3 or 4 additional animals. In about half of the experiments in which tumor-bearing animals served as the source of the transplanted tissue, the spleen was also minced and injected into several animals.

Out of 62 groups of recipient mice injected with liver from C3H animals bearing methylcholanthrene tumors, tumors arose in one or more animals in 7 groups (or in 11 per cent of the groups). In 3 of the 7 groups giving positive results, all surviving recipient animals developed tumors; in 2 groups, 4 out of 5 surviving animals developed tumors; in the remaining 2 groups, approximately half of the animals developed tumors. With but one exception the tumors were detectable within 3 weeks; the exceptional one was

* This investigation was aided by a grant from the International Cancer Research Foundation.

† Read at the 34th Annual Meeting, American Association for Cancer Research, Inc., Chicago, Illinois, April 15, 1941.

[1] Approximately one third of the liver of the host was hashed and thoroughly ground in fine sand. Five cubic centimeters of Ringer's solution was added to the macerated tissue and the mixture stirred for 5 minutes, then allowed to stand an additional 10 minutes. The suspended cells and debris were removed by centrifugalization (2,500 r.p.m. for 20 min.). The recovered supernatant fluid (4-5 cc.) was drawn into a 5 or 10 cc. syringe and filtered through a ½ inch Seitz disc (B-D No. F D) used in a Swinney B-D filter adapter (No. 423 F A). The filtrate was passed through a second disc to insure complete removal of the cells. Approximately 0.5 cc. of the filtrate was then injected into each of three or four animals.

We were unable to detect cells in the filtrate after passage through the second disc.

not observed until after 6 weeks. Tumors developed in both males and females, and in young and old animals, in approximately equal numbers.

The livers of the host animals were all found grossly and microscopically to be free of metastases. The cells, as seen in histological sections, were apparently normal. The tumors arising at the sites of injected liver were polymorphous cell fibro-sarcomas similar microscopically to the induced tumors of the hosts.

In several instances of successful tumor growth following injection of liver from C3H mice into C3H recipients, growths were not obtained when the recipients were C57 black mice. Furthermore, the livers of C57 black mice bearing subcutaneous methylcholanthrene tumors failed to produce growths in either C57 or C3H recipients.

In no instance did tumors develop from the injection of cell-free filtrates, or from minced spleen. Neither did tumors develop at the site of injection of liver from a normal animal nor from a methylcholanthrene injected animal which had not yet developed an induced tumor.

Assuming the possibility of the transference of tumor cells, which admittedly is the most plausible explanation for the developing tumors despite repeated failure to find such liver metastases, the unusually rapid development of these tumors is a feature which warrants further study. The small amount of tumor tissue which might conceivably be injected appears to have produced results out of proportion to those which occurred when a mixture of 1 part of minced primary tumor and 9 parts minced normal liver tissue was injected under similar conditions.

Summary

Freshly excised liver tissue, apparently normal and free from metastases, from mice bearing tumors induced by injections of methylcholanthrene was injected subcutaneously into normal mice. Tumors arose at the site of injection in a number of animals. Cell-free filtrates of the same liver tissue, injected in the same manner, did not induce the formation of tumors.

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Transamination in Tumors, Fetal Tissues, and Regenerating Liver*

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von Euler and co-workers (22), on the basis of results of essentially qualitative experiments, first reported that the transaminase activity of tumors was low. These investigators, using Jensen sarcoma and normal muscle, measured the rate of disappearance of oxaloacetic acid in the following reaction:

1) l(+)-glutamic acid + oxaloacetic acid $\underset{b}{\overset{a}{\rightleftharpoons}}$ α-ketoglutaric acid + aspartic acid

In addition to studies of reaction 1 in tumors, Braunstein and Azarkh (2) studied the reaction:

2) glutamic acid + α-keto acid $\underset{b}{\overset{a}{\rightleftharpoons}}$ α-ketoglutaric acid + amino acid

The amino acids investigated in the case of reaction 2b were the d- and l-forms of alanine, valine, leucine, and isoleucine. The rate of reaction 2a in which both d(−)- and l(+)-glutamic acid were used, plus pyruvic acid, was also studied. Braunstein and Azarkh (2) reported very low rates of transamination in a series of tumors, in some instances finding no evidence of transamination, and in the others, usually less than 15 per cent in 2 hours. These findings were interesting since Braunstein and Azarkh reported relatively fast rates for the same reactions in normal tissues. However, it has been recently shown (8) that the rates of reactions 2a and 2b are very slow in normal rat tissues, and that quantitatively the chief transamination substrates are those shown in reaction 1a.

Since some metabolic characteristics of tumors are also shared by embryonic tissue, it appeared of interest to study transamination in such tissues. In addition, regenerating liver was studied, since this represents a rapidly growing tissue and thus might be expected to show some metabolic similarity to tumor and embryonic tissue.

In this paper experiments are reported in which the rates of reactions 1 and 2 were studied quantitatively in 6 different mouse tumors. The rate of transamination with reaction 1a was also investigated with fetal, kitten, and adult cat tissues, in addition to regenerating rat liver.

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Tissue Sources

Tumors.—The mouse tumors employed in this investigation were the following:

1. United States Public Health Service No. 17—originally described as a neuroepithelioma (20).[1] 2. Sarcoma 37.[1] 3. Yale No. 1—an estrogen-induced mammary adenocarcinoma (5).[1] 4. No. 15091-A—spontaneous mammary medullary adenocarcinoma (6).[1] 5. No. 42—glioblastoma multiforme.[2] 6. No. 108—rhabdomyosarcoma.[2]

The tumors were transplanted at regular intervals to insure a uniform supply.

Fetal, kitten, and adult cat tissues.—Two pregnant cats provided the fetal tissue. The length of the pregnancy was uncertain, but was estimated to be in the last trimester in both instances. Hemihysterectomies were performed under nembutal anesthesia and 2 fetuses removed from each animal. The mothers were allowed to recover. One animal aborted the following day. The other animal delivered what appeared to be full term kittens 8 days later. These were allowed to nurse for 6 days.

Regenerating rat liver.—Seven albino rats, 150 to 180 gm. in weight were partially hepatectomized under ether anesthesia (11). In all instances, 60 to 70 per cent of the liver was removed. The excised tissue was used for control determinations of transaminase activity. The animals were then allowed to survive for varying lengths of time, and the transaminase activity measured in the regenerating liver.

Transaminase activity in different species.—The transaminase activity of liver, kidney, and skeletal muscle of mouse, rat, and cat are of the same order of magnitude; the per cent transamination in liver of

[1] Transplants of tumors No. 17, sarcoma 37, Yale No. 1 and 15091-A were obtained from Dr. M. Belkin, Department of Pathology, Yale University School of Medicine. The authors are indebted to Dr. Belkin for his assistance in providing these transplants and suitable strains of mice for carrying all these tumors.

[2] Transplants of tumors No. 42 and No. 108 were obtained through the kindness of Drs. H. M. Zimmerman and H. Arnold, of the Department of Pathology, Yale University School of Medicine. These tumors were originally produced by intracranial implantation of pellets of methylcholanthrene.

these 3 species under the same experimental conditions was found to be rat, 50; mouse, 46; and cat, 47.

Methods and Procedures

Experimental procedure.—The tissues were removed from the animals after exsanguination and kept in ice-cold saline. The tumors were dissected free of necrotic tissue. It was usually necessary to pool several tumors for a given experiment. Suitable samples were then weighed, after drying on filter paper, and homogenized (18) with M/10 phosphate buffer, at pH 7.4. This procedure was carried out at ice bath temperature. Aliquots of the homogenized preparations were then pipetted into the reaction flasks. All experiments with tumor tissue were carried out under anaerobic conditions, maintained by means of nitrogen gas and yellow phosphorus, in order to rule out aerobic reactions. In the case of the other tissues, anaerobic conditions were not necessary since tissue dilutions of 1:80 were used. All incubations were carried out at 38° C. with shaking.

Analytical methods.—In the experiments with the tumors, α-ketoglutaric acid was determined by the method of Krebs (15), pyruvic acid according to Westerkamp (23), and glutamic acid by a procedure previously described (4). The successful application of these analytical procedures to the measurement of transamination has been previously reported (5-7). In the case of the other tissues, reaction 1a was followed by measuring aspartic acid formation (6).

Substrates.—The substrates were all brought to pH 7.4 before addition to the homogenized tissue, with the exception of the amino acids used in the experiments shown in Table IV. In these experiments the amino acids were weighed directly into the cups, the necessary amount of alkali, or acid, was added to neutralize, and then M/3 phosphate buffer, pH 7.4, added to make up a volume of 0.3 ml. The final concentration of the substrates added was 0.016 M for each, except in the case of dl-methionine, which was 0.032 M. In the remaining experiments the substrate concentration was 0.014 M.

The source and preparation of the different substrates has been previously reported (6).

Results with Tumors

Transamination with reaction 1a.—In Table I typical results are presented for α-ketoglutaric acid formation by tumors according to reaction 1a. The per cent transamination values for the 6 tumors are considerably below those found for normal tissues, with the exception of testis, lung, and spleen (8), but indicate that at high tissue concentrations (1:10) tumors show a measurable transaminase activity when glutamic acid plus oxaloacetic acid serve as substrates.

Previous studies with purified transaminase (7) showed that the per cent transamination varied with the square root of the enzyme concentration. On this basis it would be expected that if a large amount of transaminase were present in a tissue, serial dilution would not show this relationship until a concentration of transaminase was reached which was just sufficient to catalyze reaction 1a at optimum speed. Below that concentration the per cent transamination would be expected to fall according to the square root relationship. However, if the transaminase content of the tumors was low to begin with, it would be expected that in addition to an initial low rate, the per cent transamination would decrease with dilution by an amount determined by the square root of the dilution factor. In Fig. 1 dilution curves are shown for the different tumors. The low initial rate and the rate of decrease of per cent transamination with dilution is seen to follow the square root relationship, indicating an initially low transaminase content. In contrast to this, most normal tissues show a much higher transaminase content (8).

Table I: α-Ketoglutaric Acid Formation from l(+)-Glutamic Acid and Oxaloacetic Acid

Tumor	Microliters α-ketoglutaric acid found: l(+)-Glutamic	Oxaloacetic	l(+)-Glutamic plus oxaloacetic		Per cent transamination
No. 42	105	54	670	471	35
No. 15091-A	72	41	478	365	27
No. 17	50	22	443	371	28
Yale No. 1	77	39	576	460	34
S-37	98	41	624	485	36
No. 108	65	52	514	397	30

Each flask contained 3 ml. of homogenized tumor, dilution 1:10, 0.3 ml. of 0.2 M l(+)-glutamic acid, and 0.3 ml. of 0.2 M oxaloacetic acid added as indicated. Incubation time, 15 minutes; N_2; yellow P; 38° C.

Transamination with reaction 2a.—The per cent transamination with reaction 2a, in which the α-keto-acid was pyruvic acid, is shown for the different tumors in Tables II and III. The data shown in Table II are from experiments in which the disappearance of pyruvic acid was measured. As can be seen, there is no appreciable transamination with this system even after 60 minutes incubation and with a tissue concentration of 1:10. Some of these experiments were repeated and α-ketoglutaric acid formation was measured. As seen from Table III, in confirmation of the

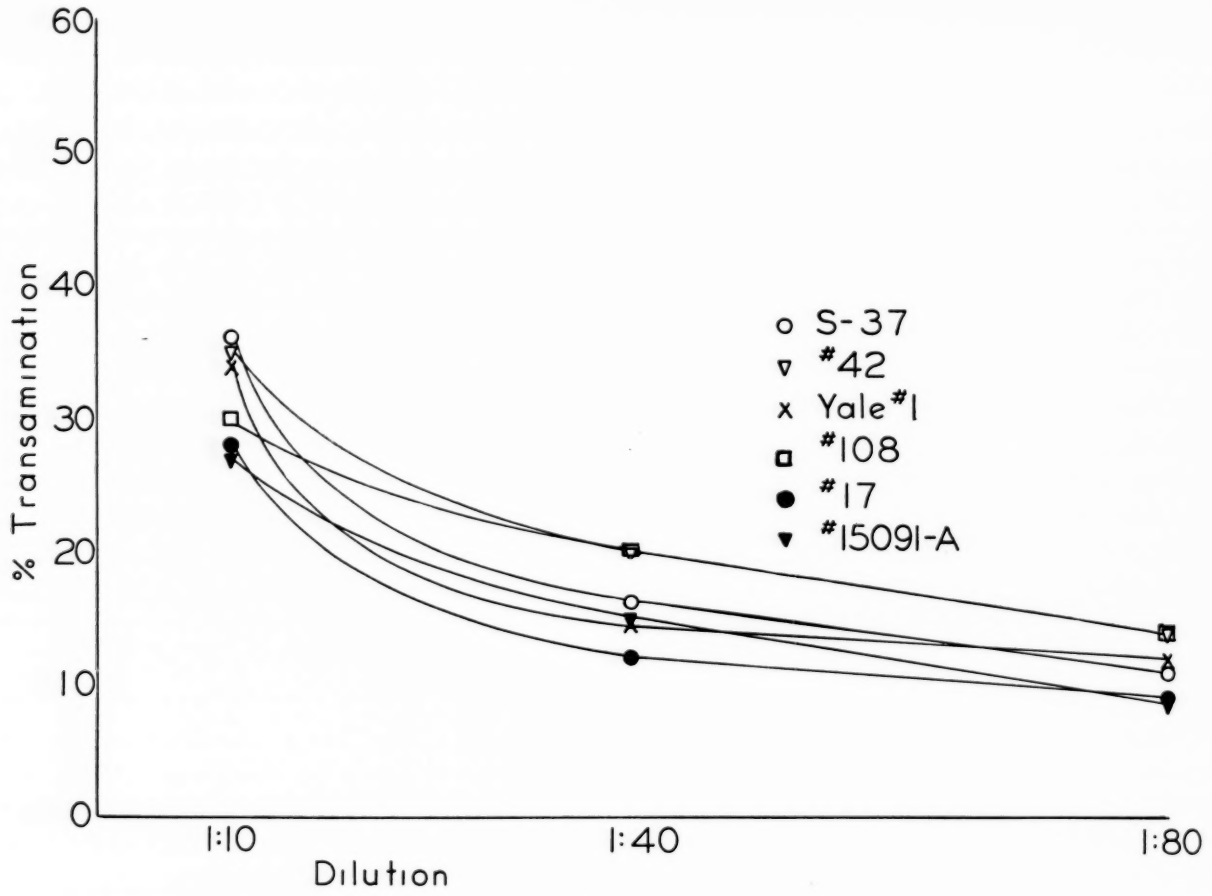

FIG. 1.—Transaminase activity of tumors at different dilutions. Substrates, l(+)-glutamic acid plus oxaloacetic acid 0.014 M. Incubation time, 15 minutes; N_2; yellow P; 38° C.

data in Table II, there is no significant formation of α-ketoglutaric acid.

TABLE II: TRANSAMINATION WITH L(+)-GLUTAMIC ACID PLUS PYRUVIC ACID (PYRUVIC ACID DISAPPEARANCE)

Tumor	Pyruvic acid found after incubation: Pyruvic acid, microliters	Pyruvic acid plus l(+)-glutamic acid, microliters	Δ microliters	Per cent transamination
No. 42	1,285	1,240	45	3
No. 15091-A	1,250	1,235	15	1
No. 17	1,330	1,300	30	2
Yale No. 1	1,270	1,225	45	3
S-37	1,260	1,215	45	3
No. 108	1,300	1,275	25	2

Each flask contained 3 ml. of homogenized tumor, dilution 1:10, plus 1 ml. of 0.06 M pyruvic acid, 0.3 ml. of 0.2 M l(+)-glutamic acid added as indicated. N_2; yellow P; 38° C.; incubation time, 60 minutes.

In normal tissues, reaction 2a proceeds at a slow but measurable rate (8) when compared to reaction 1a. In the case of these tumors it is apparent that reaction 2a does not take place at all, while reaction 1a proceeds at a much slower rate than in normal tissue.

Transamination with reaction 2b.—The formation of glutamic acid from α-ketoglutaric acid and different amino acids was determined in 3 mouse tumors. As can be seen from Table IV, the glutamic acid formation is considerable only in the case of aspartic acid. When compared with most normal tissues (8), the activity of reaction 1b in these tumors is also low.

TABLE III: TRANSAMINATION WITH L(+)-GLUTAMIC ACID PLUS PYRUVIC ACID (α-KETOGLUTARIC ACID FORMATION)

Tumor	Microliters α-ketoglutaric acid found: l(+)-Glutamic acid	l(+)-Glutamic acid plus pyruvic acid	Δ	Per cent transamination
No. 17	66	97	31	2
No. 42	74	90	16	1
No. 108	44	77	33	2

Each flask contained 3 ml. of homogenized tumor, dilution 1:10, 0.3 ml. of 0.2 M l(+)-glutamic acid and 0.3 ml. of 0.2 M pyruvic acid added as indicated. N_2; yellow P; 38° C.; incubation time 60 minutes.

Since reactions 2a and 2b have been shown to take place at very slow rates in normal tissues (8), it is not surprising to find that these reactions do not proceed

to any appreciable extent in tumor tissue, inasmuch as the fastest reaction in normal tissues, reaction 1a, has been shown to take place at a slow rate in tumor tissues.

Transamination with nonnatural amino acids.—Braunstein and Azarkh (2) reported that d-amino acids show some activity with tumor tissue in the presence of α-ketoglutaric acid. However, the analytical method employed by these workers was not satisfactory for measuring small amounts of glutamic acid as pointed out by Zorn (24). Inasmuch as reactions 2a and 2b have been shown in this study not to proceed at a significant rate with the l-amino acids it would hardly be expected that the d-forms would be active. However, since reaction 1a has been shown to proceed at a measurable rate in tumors, it seemed that d(−)-glutamic acid would be more suitable for studying transamination of d-amino acids. von Euler and coworkers (22) using qualitative tests previously reported that d(−)-glutamic acid was transaminated more slowly with oxaloacetic acid in Jensen sarcoma than in normal muscle.

Table V: Transamination of d(−)-Glutamic Acid

Tumor	Microliters α-ketoglutaric acid found: d(−)-Glutamic	Oxaloacetic	d(−)-Glutamic plus oxaloacetic
S-37	73	42	85
No. 42	66	63	102
No. 15091-A	63	76	96

Each flask contained 3 ml. of homogenized tissue, dilution 1:10, 0.3 ml. of 0.2 M d(−)-glutamic acid and 0.3 ml. of 0.2 M oxaloacetic acid added as indicated. Incubation time, 60 minutes; 38° C.; N_2; yellow P.

Quantitative measurements of this reaction are shown in Table V. As will be noted, in none of the 3 tumors studied was there any evidence of transamination with d(−)-glutamic acid. This finding is in keeping with previous experiments with purified transaminase preparations (6) and pigeon breast muscle (5).

Results with Cat Tissues

Transamination in fetal, kitten, and adult cat tissues.—In Fig. 2 data on transaminase activity, as measured by reaction 1a, are presented for fetal, kitten, and adult cat tissues. The fetuses were removed by hemihysterectomy 8 days *prepartum,* as determined by the date of delivery of the remaining fetuses. The newborn kittens were allowed to nurse for 6 days before they were killed. The mother cats served as the sources of adult cat tissues. Transaminase activity was measured in kidney, liver, and brain.

As can be seen from Fig. 2, the per cent transamination rapidly rises from fetal to adult tissues. Liver shows the greatest increase. The adult cat values for liver are of the same order as those for rat, while those for kidney and brain are somewhat lower (8). The per cent transamination values for fetal cat tissues are of the same order of magnitude as those found for mouse tumors.

Table IV: Glutamic Acid Formation from α-Ketoglutaric Acid and Different Amino Acids

Amino acid	Glutamic acid found, microliters: No. 108	Yale No. 1	No. 15091-A	Increase due to added amino acid, microliters: No. 108	Yale No. 1	No. 15091-A	Per cent transamination: No. 108	Yale No. 1	No. 15091-A
None	165	131	121	...	...	...	..	..	..
l(−)-Aspartic acid	357	273	270	192	142	149	14	11	11
l(+)-Alanine	205	167	191	40	36	70	3	3	5
l(−)-Phenylalanine	201	130	81	46	...	...	3	..	..
dl-Methionine	140	124	145	...	...	24	..	..	2
l(+)-Arginine	270	81	136	42	...	15	3	..	1
l(+)-Tryptophane	147	94	122	...	...	1	..	..	..

Each flask contained 3 ml. of homogenized tumor, dilution 1:10, plus 0.3 ml. of 0.2 M α-ketoglutaric acid. Amino acids weighed into flasks in amounts equivalent to 0.3 ml. of 0.2 M (0.4 M for dl-amino acids) solutions. Incubation time 60 minutes; N_2; yellow P; 38° C.

Transamination in regenerating liver.—Data for the transaminase activity of regenerating liver are shown in Table VI. Transamination in these experiments was measured with the substrates l(+)-glutamic acid plus oxaloacetic acid (reaction 1a). As can be seen from the data, there is a significant fall in per cent transamination between 5 and 9 days after partial hepatectomy, after which the per cent transamination returns to a normal value.

Table VI: Transamination in Regenerating Rat Liver

Days after partial hepatectomy	Mgm. dry weight of liver	Per cent transamination	$_{9}$transamination
0	11.5	52	243
2	10.2	40	212
5	10.0	39	210
9	11.2	35	168
12	11.5	51	238
24	11.6	53	245

Substrates, l(+)-glutamic acid plus oxaloacetic acid (equimolar concentration, 0.014 M). Tissue dilution, 1:80; incubation time, 15 minutes; air; 38° C.

The Qtransamination values are also decreased significantly, indicating that the fall is not due to an increase in water content of the livers. These experiments, while not striking, fall in line with those for tumor and fetal cat tissues, and suggest that where growth is taking place rapidly, the transaminase activity is decreased.

TABLE VII: QTRANSAMINATION

Tumors		Fetal tissues (cat)		Adult tissues (cat)	
S-37	57	Kidney	78	Kidney	220
No. 42	72	Brain	77	Brain	210
Yale No. 1	54	Liver	64	Liver	230
No. 15091-A.	54	Placenta	94		
No. 108	72				
No. 17	53				

FIG. 2.—Transaminase activity in fetal, kitten, and adult cat tissues. Substrates, l(+)-glutamic acid plus oxaloacetic acid, 0.014 M. Tissue concentration 1:80; incubation time, 15 minutes, air; 38° C.

Q*transamination*.—The rates of transamination in the different tissues can best be compared in terms of Qtransamination.

$$Q_{transamination} = \frac{microliters\ of\ substrate\ transaminated}{mgm.\ dry\ weight \times hours}$$

In Table VII are listed Qtransamination values for mouse tumors, fetal cat, and adult cat tissues. It is seen that the Qtransamination values for the fetal and tumor tissues are of the same order of magnitude. However, the values for the adult tissues are several times greater than those for the corresponding fetal tissues. The Qtransamination value for regenerating rat liver 9 days after partial hepatectomy (Table VI) is of the same order of magnitude as that of kitten liver, but apparently never reaches the low value of fetal cat liver.

DISCUSSION

Tumor, fetal cat, and regenerating liver tissue have in common the property of rapid growth, or rapid protein synthesis. These tissues, particularly the first two, also show a low transaminase activity. This would suggest that an inverse relationship exists between protein synthesis and transaminase activity. The exact mechanism by which the transamination reaction could influence protein synthesis is not clear. However, if one considers the substrates involved in reaction 1, it is seen that we are dealing with substances which are known to play special roles in intermediary metabolism. Thus, oxaloacetic acid and α-ketoglutaric acid act as respiratory mediators in the Szent-Gyorgyi-Krebs cycle (16). On the other hand, glutamic acid and aspartic acid play important roles in protein metabolism. Schoenheimer and Rittenberg (19) have

stated, "The results obtained with N^{15} strongly support the theory that the dicarboxylic acids play a central role in protein metabolism." These workers observed that whenever isotopic amino acids, or ammonia, were fed, the dicarboxylic acids showed a much higher isotope concentration than the other amino acids, with the exception of the amino acid fed. Glutamic acid always showed a higher concentration of isotope than aspartic acid.

While the facts are too few to permit a satisfactory formulation of the mechanism by which transamination influences protein synthesis, for purposes of a working hypothesis the interrelationship might be pictured as follows. Glutamic acid plays a central role in protein synthesis. From the work of Schoenheimer and Rittenberg (19) it is clear that the protein molecule cannot be considered as a stable substance, but rather as a highly reactive compound which continuously participates in exchange reactions with free amino acids. Thus the protein molecule can be pictured as existing in a dynamic equilibrium with free amino acids and other metabolites. In the presence of high concentrations of transaminase, the amount and rate of protein synthesis would be determined by the rate at which glutamic acid is "pulled out" of the protein-amino acid equilibrium system. Since reaction 1a proceeds at a rate 2 to 3 times as fast as reaction 1b (7, 8), aspartic acid synthesis would take place at the expense of glutamic acid, with the result that protein synthesis would be limited. The high transaminase activity of adult tissues with a slow rate of protein synthesis, and the low activity of tumor and embryonic tissues with a high rate of protein synthesis, fit into such a hypothesis.

In addition to low transaminase activity, low values for cytochrome C (12, 17, 21), coenzymes I and II (1, 14) and riboflavin (13, 14) have been reported for tumor and embryonic tissue. These deficiencies would be expected to limit the oxidative capacity of tumor and embryonic tissue and consequently would result in a more limited oxidation of glutamic acid. In the case of adult tissues, the high transaminase content would act in such a way as to "pull out" glutamic acid, while the higher content of cytochrome C, coenzymes I and II, and riboflavin would insure rapid oxidation of glutamic acid via *a*-ketoglutaric acid, succinic acid, and finally, oxaloacetic acid. The latter could then be used in transamination with another molecule of glutamic acid. The fact that of the amino acids, glutamic acid alone is rapidly oxidized by most tissues lends support to the above speculations. In the case of tumor and embryonic tissue, the low values for transaminase, cytochrome C, coenzymes I and II, and riboflavin would serve to insure a high glutamic acid content for purposes of protein synthesis.

The recent finding that tumors and embryonic tissue are low in arginase (10) may represent another mechanism by which an amino acid is "preserved," as it were, for the purpose of protein synthesis in rapidly growing tissues.

Summary

1. The transaminase activity of a series of 6 mouse tumors, regenerating rat liver, and fetal kitten and adult cat tissues was determined with the reaction:

1) l(+)-glutamic acid + oxaloacetic acid $\underset{b}{\overset{a}{\rightleftharpoons}}$ *a*-ketoglutaric acid + aspartic acid

The tumors and fetal tissues showed low activities and the regenerating liver a somewhat lowered activity when compared to normal adult tissues.

2. No transaminase activity was observed with three different tumors when d(−)-glutamic acid was used in reaction 1a.

3. The reaction:

2) l(+)-glutamic acid + *a*-keto acid $\underset{b}{\overset{a}{\rightleftharpoons}}$ *a*-ketoglutaric acid + amino acid

was also studied with different tumors. In the case of reaction 2a, with pyruvic acid as the *a*-keto acid, no measurable transamination occurred. In the case of reaction 2b, the amino acids l(−)-aspartic acid, l(+)-alanine, l(−)-phenylalanine, dl-methionine, l(+)-arginine and l(+)-tryptophane were used. The transaminase activity was appreciable only in the case of l(−)-aspartic acid (reaction 1b).

4. The suggestion from these experiments that an inverse relationship exists between transaminase activity and protein synthesis is discussed.

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Induced Resistance to a Transplantable Lymphatic Leukemia in Rats*

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Early in the investigation of transplanted mammalian tumors, it was found that a resistant state could be induced by the injection of homologous living normal cells some days prior to the cancer inoculation.

Rhoads and Miller (5) have reported that immunity to transplantable leukemia in mice could be induced by the same method. MacDowell, Taylor, and Potter (2) extending this observation found that the induced protection depended on the genetic constitution of the immunizing tissue. Embryonic tissue from a highly inbred strain of mice did not induce resistance to inoculated leukemia in the same strain but the embryonic tissue from another strain or from hybrids of the inbred strains, was highly effective. In this laboratory Barrett (1) has demonstrated a similar relationship between the genetic make up of the normal tissue used for immunization and the degree of resistance induced to transplanted mouse tumors.

The present report is on an investigation of the induced resistance to a transplantable lymphatic leukemia of the rat (4). The strain was started from an animal in which the disease had been induced by an injection of dibenzanthracene. Like certain leukemias in mice, transplants of the cells may result in typical leukemia with lymphocytosis, and extensive involvement of the thymus and lymph nodes or the manifestation of the disease may be confined to a local lymphosarcoma. The original animal in which the condition was induced came from a subline of the Wistar Institute strain of rats and this strain has proved to be highly susceptible to transplants of the leukemia cells. About 90 per cent of those inoculated die of the disease in an average time of 9 days.

* This investigation was aided by a fund for leukemia studies contributed anonymously.

Experiments

Group 1.—This group is made up of four individual experiments and as the procedure was identical they are reported together. In each experiment a number of Wistar rats of the same age were divided into three lots. One of these was injected with cells from 18 day old embryos of the Wistar strain, one lot with defibrinated blood from adults of the same strain, and the third lot was kept for controls. The immunizing injections were given both subcutaneously and intraperitoneally. Some 12 days later all were inoculated intraperitoneally with 0.2 cc. of leukemic cells. The results are given in the first section of Table I.

Table I: Summary of Experiments

Immunizing material	Amount and location of injection	No. of rats Wistar strain	No. developed leukemia	Per cent resistant
Group 1				
Embryonic tissue from Wistar strain	0.2 cc. subcutaneously 0.3 cc. intraperitoneally	44	2	95.4
Defibrinated blood from adult Wistar rats	0.2 cc. subcutaneously 0.3 cc. intraperitoneally	21	3	85.8
Controls		41	37	9.5
Group 2				
Embryonic tissue from Wistar strain	0.5 cc. intraperitoneally	30	3	90.0
Embryonic tissue from Wistar strain	0.5 cc. subcutaneously	30	2	93.6
Controls		40	33	17.5
Group 3				
Embryonic tissue from hooded rats	0.5 cc. intraperitoneally	20	0	100.0
Embryonic tissue from hooded rats	0.5 cc. subcutaneously	20	5	75.0
Controls		20	18	10.0

Group 2.—There were three individual experiments in this group. The same procedure was followed as that used for group 1. One lot in each experiment was injected intraperitoneally with cells from 14 day old embryos of Wistar strain, one lot was given the same material subcutaneously. These, with the controls, were inoculated 12 days later. The results are given in the 2nd section of Table I.

Group 3.—The immunizing material for these animals was the tissue of embryos from the Rockefeller Institute strain of hooded rats. As with the preceding experiments, one lot was injected intraperitoneally and another subcutaneously with the immunizing normal cells and these animals with controls were inoculated with leukemia cells 12 days later. The results in two identical experiments are given in the 3rd section of Table I.

It is evident from the above experiments that a high degree of resistance is induced against transplanted leukemia in rats by a prior injection of homologous normal defibrinated blood or embryonic tissue. The embryonic cells from the Wistar strain were just as effective in the production of resistance as those from another strain, the hooded rats. The results were equally as good when the immunizing injections were given subcutaneously as when given intraperitoneally. As the leukemia inoculations were made intraperitoneally, this result would indicate that the resistance depended on a general reaction rather than a local one.

Summary and Discussion

It is known that a definite resistance to transplanted leukemia in mice may be induced by an injection of embryonic tissue prior to inoculation. The results of experiments reported in this paper establish the same fact for a transplanted lymphatic leukemia in rats. This emphasizes further the similarity between transplanted leukemia and malignant tumors. The important observation of MacDowell and his associates (2) that the immunizing power of the normal tissue depends on its genetic constitution, was not properly tested in the present study. However the principal is so well established that we are probably justified in assuming that the Wistar strain used was not genetically homogeneous because the embryonic tissue from the strain was just as effective in its immunizing property as the tissue from an unrelated strain. If this induced resistance is related to a sensitization phenomenon (3), as has been suggested, the tissues from a homogenous strain would cause no greater reaction than an autograft and no sensitization would result.

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The Nonspecific Nature of Induced Resistance to Tumors

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Some forty years ago, when the systematic investigation of transplantable new growths was begun, it was noticed that animals which had been unsuccessfully inoculated with a tumor of their own species, or in which such a neoplasm had receded, generally resisted a second inoculation. Soon afterward it was shown that a similar immunity could be elicited with homologous normal tissues. Although the difference between a spontaneous new growth and one making its way in the alien soil of another animal were almost immediately pointed out, the hope of discovering an effective serum or tissue extract led to an enormous amount of labor and a flow of contradictory publications that has only just begun to slacken. As this early work has been reviewed by one of us (12) it need not be discussed here; in any case its interest is now mainly historical.

The confusing nature of the problem and the prominence which it attained are revealed in the detailed study by Russell (11), who in 1912 reviewed the concepts of his time and resolved some of the apparent contradictions that were then rife. Others could not be explained until new developments in the science of genetics had made it clear that neoplasms propagated in mixed stocks such as those formerly used elicit antibodies that are directed against them not as tumors, but merely as cells originally derived from genetically different individuals. The chief experimental evidence on this point is summarized in the following brief review of recent papers.

In 1936 Bittner (3) showed that resistance could not be produced in mice of pure strains against carcinomas originating within their own strains. Comparable results have been described recently by Barrett (1), who found that the degree of resistance induced varied with the genetic interrelationship of the host, the tumor, and the donor of the blood used for immunization. With the two pure-line neoplasms employed, no significant resistance could be aroused in inbred mice against growths originating within their own strains. The work of Lewis (8), too, on the transplantability of dibenzanthracene sarcomas produced in various inbred strains gives ample evidence that genetic differences between a tumor and the animal into which it is introduced are alone responsible for acquired immunity. Thus a sarcoma from one strain, when inoculated into mice of another, made them refractory to a second graft of the same tumor, but a transplant in mice of the line in which a new growth originated produced no resistance.

Still further proof is furnished by the work of Gorer (6) on genetically pure stocks and by that of Phelps (10), who demonstrated that the serum antibodies investigated by Lumsden (9), and ascribed to the presence of neoplasms that were from unrelated stocks, are not specifically directed against malignant cells. On the contrary, they appear to be isoantibodies formed in response to the injection of foreign homologous cells.

Despite this array of evidence to indicate that resistance is a nonspecific reaction depending upon differences in the genetic constitution of an animal in which a neoplasm originates and the animals that maintain its existence in successive generations, the Brown-Pearce carcinoma of the rabbit has gained favor as a test object in a renewed attempt to establish the specific nature of tumor immunity. Although this growth is undoubtedly of some value, because of the comparatively large size of the animal in which it is propagated and the profusion of its metastases, serious disadvantages accompany investigations based solely upon its use. It must generally be grown in nonuniform stocks which certainly are not related to the original bearer, and the percentage of successful inoculations is subject to considerable variation since partially or totally resistant animals are not infrequently encountered.

Basing their assertion upon experiments with this neoplasm, Besredka and Gross (2) stated that a preliminary intracutaneous implantation which gave rise to a receding tumor caused permanent resistance to intratesticular inoculation. This observation has been confirmed recently by Cheever and Janeway (4), but these authors regarded the immunity as not wholly specific since it could be elicited with embryo skin also. Furthermore, Jacobs and Houghton (7) found that the complement-fixing reactions described by previous investigators between saline extracts of the Brown-Pearce carcinoma and the serum of some rabbits bearing it were weak and variable, and suggested that genetic differences between neoplasm and host might be responsible for the existence of these antibodies.

Experimental

The experiments to be described in the present paper bring additional evidence that acquired resistance to propagable neoplasms is nonspecific in its nature. Advantage was taken of an ideal situation. The growth employed (R 2426) is a transplantable mammary adenocarcinoma of the rat, described pre-

viously by one of us (5), which originated spontaneously in a female of the 27th brother-by-sister generation of the August line. The high degree of homozygosity of the members of this, and the succeeding generations employed for transplantation, is revealed by the pronounced specificity of the tumor and the regularity with which its transplants proliferate. Not a single instance of failure in rats of the strain in which it arose has been recorded during the 2½ years, and 18 generations, of its propagation. On the other hand, it rarely grows in unrelated stocks.

The August strain originated from a cross between rats of an earlier August strain (line 990) and line 1561. Line 990 has since been maintained as a distinct family by continued inbreeding; line 1561, however, no longer exists.

A fair degree of success was achieved with transplants in rats of line 990, clearly indicating the maintenance of a genetic relationship between this and the August line, despite the fact that they have been inbred separately for many years. Thus of 19 rats of line 990 previously tested, 11 grew the tumor, though its proliferation was slower than in animals of the August line, in which it originated, and necrosis more pronounced. Nevertheless, the results were sufficiently different from those following transplantation into wholly unrelated stocks to suggest that this carcinoma would be useful for the experiment now about to be described.

The immunizing effect of embryo skin, a highly efficient agent when both it and the tumor against which it is employed are derived from heterozygous sources, was investigated in rats of the August line, in which the tumor originated, and in related animals of line 990. Embryos were removed aseptically toward the end of pregnancy, the skin was removed, clipped with scissors until it could be drawn with ease into a Bashford syringe, and injected subcutaneously on one side of the body in doses of 0.3 cc. Here it consistently produced a foreign body reaction which did not vary significantly with the source of the material or the strain into which it was introduced. Grafts of the tumor weighing approximately 0.003 gm. were inoculated subcutaneously into the opposite side of the body 13 days later. Both sexes were employed, and the animals were from 2 to 5 months of age when inoculated. The viability of the tumor was always tested by simultaneous transplantation into August rats.

The following combinations were employed:

1. August strain embryo skin into August strain rats; 2. Line 990 embryo skin into line 990 rats; 3. August strain embryo skin into line 990 rats.

The results are set forth in the accompanying table. It is clear that embryo skin from the August line, in which carcinoma R 2426 originated, had no power to induce resistance in rats of this strain, nor was any significant amount elicited in line 990 animals with embryo skin from line 990. But when rats of line 990 were injected with embryo skin from August rats, a high degree of resistance ensued.

In other words, embryo skin that was genetically identical with the tumor possessed the capacity to immunize against it only in rats of a line partially alien as regards both the embryo skin and the corresponding tumor.

TABLE I: EFFECT OF PREVIOUS TREATMENT WITH EMBRYO SKIN ON GRAFTS OF A CARCINOMA IN RATS OF THE STRAIN IN WHICH IT ORIGINATED AND OF A RELATED STRAIN

Number of rats	Strain of inoculated animals	Source of embryo skin	Number with tumors	Percentage of resistance
10	August	August	10	0
22	Line 990	Line 990	14 (3 R)	36.3
40 *	Line 990	August	3	92.5
41	Line 990	Untreated Controls	32 (10 R)	21.9

R = Tumors which proliferated for a time but eventually regressed.
* Because of inadequate material 6 rats of this group received 0.3 c.c. embryo liver mash, and were among those that did not grow the tumor.

SUMMARY

Rats of a highly inbred line, which are uniformly susceptible to inoculation with a mammary carcinoma that arose spontaneously in a female of this line, could not be immunized against the tumor with embryo skin from the same line, though this material was highly effective in a partially related line.

When the experiment was repeated in this partially related line with embryo skin derived from within it, no significant degree of immunity was achieved.

Thus acquired resistance to transplantable tumors depends upon genetic differences between the animal inoculated and the one that originally produced the neoplasm, and is not specifically directed against the malignant cell.

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Estrogenic Effects of Adrenal Tumors of Ovariectomized Mice*

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Certain tumors of the adrenal glands in man have been associated with evidences of masculinization. Precocious sexual development or intersexual conditions are associated with the development of adrenal tumors in young individuals (8). Androgenic substances have been obtained in large amounts from the urine of several patients with such tumors (1, 3, 5). More recently the urinary androgens of individuals with adrenal tumors have been extracted and chemically identified (3, 5). An increased amount of estrogen, presumably estrone, was eliminated in the urine of one patient with an adrenal tumor (1).

Few tumors of the adrenal glands of experimental animals have been described, especially tumors demonstrating stimulation of the genital tissues. Masculinization accompanied adrenal adenomas in old male guinea pigs which were castrated shortly after birth (13).

Mammary tumors have appeared in old female mice of the JAX Dba strain which had been ovariectomized immediately after birth (17, 18). These mice showed tumorous changes of the adrenal glands which were associated with mammary proliferation. These observations have been extended more recently to include mice of one other strain (19).

During the last few years a number of tumorous conditions of the adrenal glands have been observed in our laboratory in ovariectomized mice receiving intravaginal instillation of benzpyrene dissolved in oil, in untreated ovariectomized mice, or in mice receiving estrogens for prolonged periods. The adrenal tumors in the untreated ovariectomized mice and the associated evidence of physiological activity will be described.

Materials and Methods

Fifteen mice were ovariectomized when 43 to 65 days of age. The ovaries, ovarian capsules, uterine tubes, and upper part of the uterine horns were removed. The mice were from the third and fourth generations of the NH strain, developed by Dr. L. C. Strong by hybridizing the JK and CBAN strains. The mice of the parental strains showed a very low incidence of spontaneous mammary tumors. Three of the test animals had received one injection of a substance tested for possible estrogenic activity and found to be inactive. They were fed Purina Fox Chow and water. All of the animals were autopsied shortly after death or were killed when death was considered to be imminent. The genital tissues, adrenal and mammary glands, and skeletal tissues were examined and prepared for histological examination.

Observations

The mice of the early generations of inbreeding were not homogenous for coat color or, presumably, for other characters. The castrated mice attained weights of over 40 gm. and were obese until they became quite old. Two mice developed mammary tumors and 2 had lymphatic leukemia. At autopsy the accessory genital tissues and mammary glands were well developed in the first animal examined and both adrenals were enlarged to several times the usual size (No. 9, Table I).

The adrenal glands or tumors of the mice varied in gross appearance at the time of autopsy, depending in part on their size and, as determined later, their microscopic structure. The 3 largest tumors (Nos. 10, 23, and 25, Table I) were reddish and approximately two-thirds as large as the kidneys, weighing from 146 to 158 mgm. Their external peritoneal surfaces were smooth and they were apparently quite loosely attached to the perirenal adipose tissue. Small brownish or red spots were seen in one of the tumors. Adrenal tissue was not detected on gross examination of the larger tumors. Smaller tumors which appeared in one or both adrenals of 6 mice were irregularly shaped and adrenal cortical tissue was still recognizable (Figs. 1 and 2). The tumors of several animals in this group weighed 30 to 50 mgm. Areas of pale reddish tissue of variable sizes appeared at one or more points of the enlarged glands and brownish and light yellowish adrenal tissue could be identified at other points. The glands had irregular surfaces and were pear-shaped or irregular rather than rounded or oval as the normal adrenal gland or the largest tumors. The smaller tumors of the adrenal

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glands of 2 mice were not identified definitely before microscopic examination (Fig. 3). The adrenal glands presenting such tumors were usually spotted with pinkish, brown, and yellowish patches, had a slightly irregular surface, and were enlarged. Tumors arose in both adrenals of at least 7 mice although one tumor was usually larger than the other. When the tumors were unilateral they appeared on either side although 2 of the 3 largest were on the right.

The larger tumors were composed of cords of small, densely staining cells or follicle-like structures lined by a stratified epithelium. The cords of cells or follicles were surrounded by delicate connective tissue septa (Figs. 4 and 5). Peripherally the cords contained both the small, hyperchromatic cells and large cells with a vacuolated or clear cytoplasm. The follicle-like cavities were filled with either a coagulated material or with blood. The smaller cells resembled those in the glomerulosa of a hyperplastic adrenal cortex. Cell boundaries were usually indefinite, in this respect resembling the granulosa cells of the ovarian follicles. Hyperplasia was not uniform throughout the tumors. Some fields observed at high magnification showed numerous mitotic figures while few dividing cells were found in other regions. Fragments of normal cortical tissue were detected at one or more comparatively limited areas at the periphery of the larger tumors. Although a definite capsule was not present, invasion by the larger tumors of the surrounding tissue was limited. The tumors of moderate size (Figs. 1 and 2) were more definitely circumscribed although they had destroyed the greater part of the normal adrenal tissues and had greatly enlarged the adrenal structure. The smaller tumors were more or less circumscribed in the cortical tissues as well as by the surrounding tissue. In the smaller tumors, cords of large vacuolated cells intermingled with the peripheral cords and islands of the smaller hyperchromatic tumor cells. Occasionally small masses of brownish cellular material (brown degeneration) also intermingled with the hyperchromatic cells (Figs. 3 and 4).

The number of small tumors was insufficient to permit a definite determination of their site of origin. The smaller lesions (Fig. 3) involved the entire thickness of the adrenal cortex, and although their cells resembled those of the glomerulosa, which may be quite thick in mice, the present observations merely indicate a glomerular origin. Around some of the adrenal glands containing the smaller tumors nodular areas were observed which apparently had penetrated the adrenal capsule and pushed out into the perirenal fat. These nodules contained irregular cords of cells apparently undergoing fatty degeneration with loss of cellular detail. This might indicate regression of the smaller tumors which formerly contained a preponderance of large vacuolated cells.

Careful examination at autopsy of the regional nodes and lungs failed to disclose metastatic tumors.

The accessory genital organs presented varying states of hypertrophy or regression closely paralleling the

TABLE I: ADRENAL TUMORS IN OVARIECTOMIZED MICE

Mouse No.	Age castrated, days	Age at death, days	Uterus	Interpubic ligaments, mm.	Adrenal tumors	Other tumors
1	43	603	Pyometra	Moveable	2 medium tumors	
2	43	696	Cystic glands	Moveable	2 small tumors	
3	43	691	Proestrous	Moveable	1 small tumor	
6	65	672	Cystic glands	1	1 medium tumor (Fig. 2) 1 small tumor	Mammary
7	43	619	Cystic glands	....	2 medium tumors (Fig. 1)	
8	43	619	Cystic glands	2	1 medium tumor	
9	43	495	Small hyalin	2	2 medium tumors	
10	43	619	Marked hyalin	2	1 large tumor	
12	65	618	Atrophic	....	Cortex irregular	
14	65	694	Very large cystic gland	2	2 medium tumors	
15	65	649	Atrophic	....	Cortex irregular	Lymphoid
22	65	674	Cystic glands	1.5	1 small tumor (Fig. 3)	Lymphoid
23	60	724	Cystic glands	....	1 large tumor 1 small tumor	 Mammary
24	60	726	Cystic glands	1	1 small tumor	
25	60	719	Cystic glands	2	1 large tumor 1 small tumor	

condition of the adrenal glands. The mammary glands were all developed more extensively than those usually observed in castrated mice. The mammary glands were especially well developed in the mice with both medium sized and larger adrenal tumors. The ducts were large, and many small branches and alveoli were present (Fig. 7). The glands of two mice contained localized alveolar nodules. The ducts contained concretion-like secretory products similar to those found in the glands of mice subjected to prolonged estrogenic treatment. Two mice had mammary adenocarcinomas.

The uteri usually resembled those of mice which had received variable amounts of estrogen for prolonged periods. Some were large and contained many distended and cystic glands (Fig. 6). One mouse died with pyometra. One mouse had small uterine horns and the stroma of the endometrium showed marked hyaline degeneration. All of the mice with the larger tumors had a thick stratified or cornified vaginal epithelium (Fig. 8). The cervices were greatly enlarged by a hypertrophy and mucoid-like transformation of the stroma.

The separation of the pubes at the symphysis by an interpubic ligament also indicated the influence of estrogenic hormone. The femurs of two of the mice contained numerous osseous spicules in the medullary cavities, and the diaphyseal walls were thickened.

Discussion

The relationship between the gonads and adrenal glands has been indicated in several ways. The clinical picture accompanying adrenal tumors may indicate abnormal stimulation by gonadal hormones. The administration of gonadal hormones and at least some of the adrenal hormones has revealed some overlapping of physiological activities (15). The injection of desoxycorticosterone results in the formation of a progestational endometrium in rabbits when preceded by estrogen and in the appearance of the copulatory response in guinea pigs (15). The above tissue or animal reactions were also induced by progesterone. Desoxycorticosterone will also induce mammary growth in mice (16). On the other hand, several sex hormones have effects qualitatively similar to some adrenal hormones on salt metabolism (14). The gonadal hormones, especially the estrogenic, induce changes of the pituitary glands, which in some species are manifest both histologically and functionally (6). The removal of the gonads increases the gonadotropic hormone in the hypophysis. The influence of chronically administered estrogens upon the degranulation of the chromophilic cells of the hypophysis, in rats and mice, also indicates a reciprocal hypophysial-gonadal relationship. Hypophysial tumors arise in such animals although other factors are probably also involved (8). In mice of at least one strain interstitial cell tumors of the testis appear in estrogen-treated mice (7, 10), although prolonged estrogenic stimulation more typically leads to regression of both the glandular interstitial cells and the seminiferous epithelium.

In common with the influence of estrogens on the development of hypophysial and testicular tumors an explanation of the mechanism contributing to the proliferation of the adrenal glands with the formation of tumors following castration is not available. That they result from a hormonal disturbance or lack of balance is entirely probable but not especially informative.

The autonomy of the adrenal tumors has not been proved by transplantation into genetically related hosts in various physiological states. Some tumors, malignant in some respects, are capable of progressive growth in closely related hosts only when these hosts are properly adapted physiologically. Transplantation was not attempted in the present experiments because the heterozygous nature of the animals would probably have led to negative results even with tumors unquestionably malignant.

Adrenal tumors in ovariectomized mice have been uniformly associated with evidence of estrogenic stimulation of their hosts. In the present experiments the size of the tumor could be roughly correlated with the evidences of the high or low levels of estrogenic stimulation. That the endocrine activity was exerted by, or at least associated with these tumors, was indi-

DESCRIPTION OF FIGURES 1 TO 5

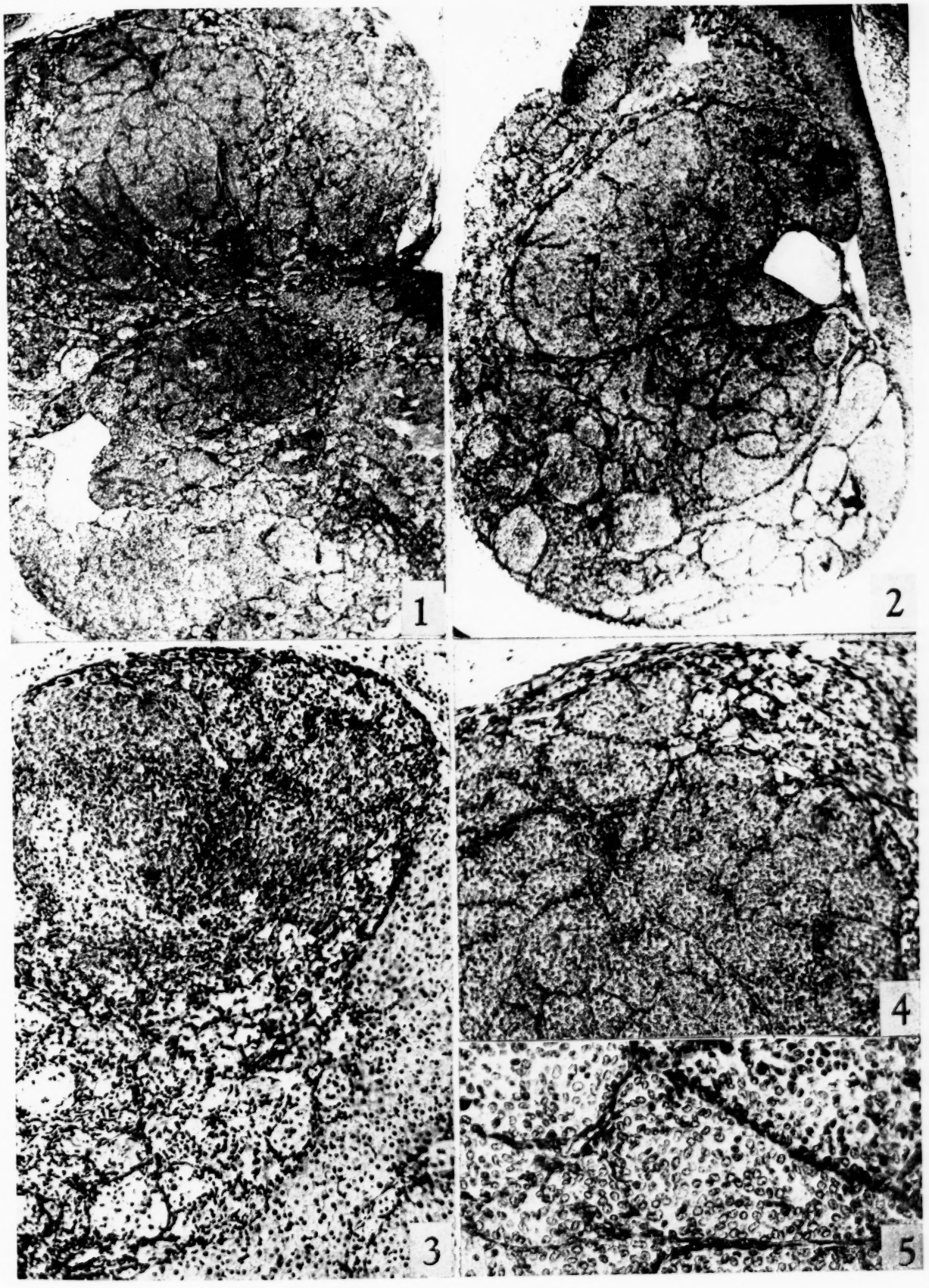
Fig. 1.—Tumor of the adrenal gland of a mouse (No. 7, Table I). The central part consists largely of cords of small hyperchromatic cells. At the periphery the cords contain vacuolated cells. This section contains no medullary tissue and no normal cortical tissue. Mag. × 30.

Fig. 2.—Tumor of the adrenal gland of mouse No. 6 (Table I). The adrenal was pear-shaped, the tumor occupying one pole. A rim of normal cortical tissue is on the right and upper end. The medulla is seen in the upper center. This tumor consisted largely of cords and masses of small cells resembling those in the glomerulosa. Mag. × 30.

Fig. 3.—Small tumorous nodule of adrenal of mouse No. 22 (Table I). The small nodule bulges against the capsule. A sharp border exists between the nodule and normal cortex. At the lower portion, the small cells blend with cords containing larger vacuolated cells some of which contain brownish pigment. Mag. × 90.

Fig. 4.—Area of tumor shown in Fig. 1. Mag. × 90.

Fig. 5.—Area of tumor shown in Fig. 2. Mag. × 180.



FIGS. 1 TO 5

cated by the condition of all the accessory genital tissues and the skeletal tissues. The dissolution of the pubic symphysis, for example, was inhibited by adequate testosterone even when estrogens were administered. The cystic uteri, enlarged cervices, well developed mammary glands containing intraductal concretions and nodules have not accompanied the injection of androgens.

The appearance of mammary adenocarcinomas in two of the mice and of lymphoid tumors in two other animals provides little significant material for interpretation since control animals maintained on the same diet are not available. On another diet, mammary or lymphoid tumors occurred rarely in NH mice of these particular generations. All hormones which have been capable of inducing and maintaining adequate mammary development in male mice from strains with an appreciable susceptibility to mammary tumors have been followed by mammary neoplasia. It seems possible that intrinsic hormones capable of eliciting mammary growth, whether produced by the hyperplastic or neoplastic adrenal tissue or the ovary, might result in mammary tumors in the proper animals. The point of major interest is not that mammary tumors appeared, but that adrenal neoplasia associated with evidences of estrogenic stimulation were acquired by ovariectomized mice, in this instance from a stock of presumably low susceptibility to mammary tumor.

Summary

Tumors of the adrenal glands arose in 13 of 15 ovariectomized mice of the early generations of the NH strain. The tumors weighed from 158 to 146 mgm. in 3 mice. The adrenal glands of 6 mice were two or more times the usual dimensions; in some animals they weighed 30 to 50 mgm. The tumors consisted of cords or, in one case, follicular structures of small hyperplastic cells. The smaller tumors showed some indication of cellular differentiation, since large vacuolated cells frequently intermingled with the cords or islands of smaller cells.

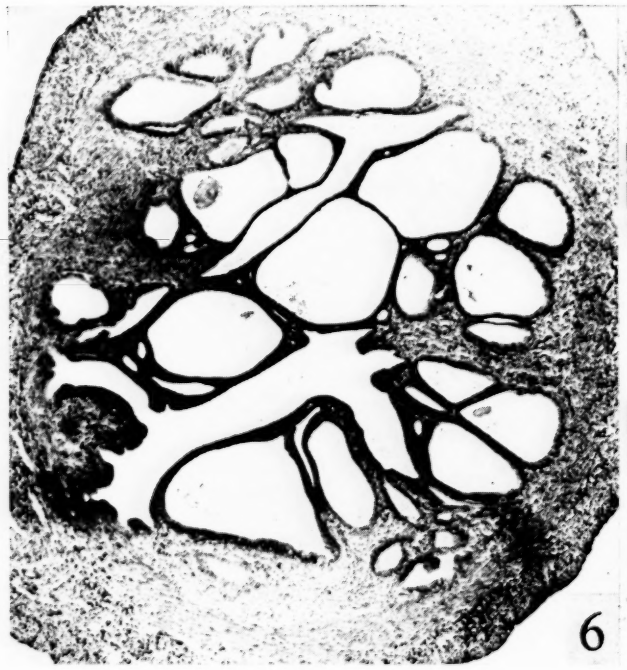


Fig. 6.—Cross-section of the uterus of mouse No. 7 (Table I) showing cystic glandular hyperplasia. Mag. × 30.

Fig. 7.—A mammary gland of mouse No. 9 (Table I) showing external development of the mammary ducts and small alveoli.

Fig. 8.—A section of the vagina of mouse No. 14 (Table I). The vaginal epithelium was thickly stratified although not cornified. Mag. × 30.

These adrenal tumors were associated with evidences of estrogenic stimulation as determined by the condition of the uteri, pelves, and mammary glands.

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Occurrence of Estrogenic Hormone in Ovarian Cysts*†

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Relatively little is known concerning the genesis of ovarian tumors and the etiologic relationship of the benign to the malignant forms. The various endocrine components of the ovary and the many hormonal influences which affect its development suggest that hormones may play a role in the production of ovarian tumors. Excessive amounts of gonadotropic hormone in both the human and in the laboratory animal may stimulate changes in the ovary, which although not neoplastic, nevertheless may be pathologic in nature. Similar conditions such as "cystic ovaries," and follicle and corpus luteum cysts, occur spontaneously in the human. That hormones play a part in some types of ovarian tumors is shown with certain rare tumors by clinical manifestations, excretion of unusual amounts of hormone, and occurrence of large amounts of hormone in the tumor tissues and fluids.

For many years the consideration of the effect of hormones on the production and development of tumors has been the subject of extensive studies both in the clinical and experimental fields. These studies have been concerned with hormone imbalance at physiologic levels, e.g., castration, pregnancy, etc., and at excessive levels by the administration of hormones. The recent studies on the relation of sex hormones to the production of benign and malignant tumors make it timely to consider any possible relation of estrogenic hormones to ovarian tumors.

Since estrogenic hormone occurs naturally in normal ovarian tissue (2, 3) it is of interest to investigate the occurrence of this hormone in ovarian tumors, especially the commonly occurring types. Excluding the previous report of the authors (1) more than 100 ovarian cyst fluids (3-5, 7-17) have been tested for estrogenic hormone. Some of the reports have been incidental to other studies but others have attempted to correlate the hormone finding with the histology of the tumor. Unfortunately most of the reports lack data either on the number of tumors examined, their size, the amount of fluid tested, the method of assay or the histology of the tumor. With the exception of an early report (4) on 3 unclassified ovarian cysts the fluids have been tested only in amounts which were convenient without extraction. Although these small amounts were usually sufficient to detect the presence of hormone in follicle and corpus luteum cysts (3, 5, 7, 9, 10, 12-15, 17) they were not adequate to establish the absence of the hormone in other types of cysts. With the amounts of fluid tested estrogenic hormone was found in 5 serous cysts (7, 9, 10) and 1 pseudomucinous cyst (12). The hormone was not found in fluids of 3 malignant ovarian cysts (9, 13). Similarly, parovarian (6, 7, 9, 13) and other extra-ovarian cysts (12, 13) did not contain the hormone.

This study is a continuation of a previous report (1) on the occurrence of estrogenic hormone in human ovarian tumors. It was hoped that a correlation of the hormone findings with the histology of the tumor might throw some light on the role of hormones in these tumors. This report deals only with the commonly occurring ovarian tumors. Tumors associated with pregnancy and the puerperium have been excluded from this report because of the large amounts of estrogenic hormone in the maternal circulation during pregnancy and the possible effects of the pregnancy hormones upon the development of the tumor. Because of the multiplicity of histologic components of the ovary and of the tumor an attempt has been made to simplify the problem by confining the study to the cystic tumors in which each cyst may be considered as a histologic entity with specific cellular lining. Extraction of the fluids has permitted large amounts of fluid to be tested.

Methods

Clinical data.—These data include only the cystic ovarian tumors and exclude all rare tumors. Except for dermoids which are often solid or semisolid, the series is fairly complete. The ovarian cysts associated with pregnancy and the puerperium have been placed in a separate group because of different hormonal conditions and these results will be reported later. The clinical material has been obtained from the University of Chicago Clinics and the Chicago Lying-in Hospital and entirely from white women. For completeness the data previously reported are included in this report.

General.—The ovarian cysts were received immediately after removal and the fluids aspirated immediately to prevent any possible diffusion of hormone from adjoining ovarian structures. A representative specimen of tissue was selected from the cyst wall, care being taken to avoid adjacent structures which might

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confuse the histologic picture. When more than one cyst was present, each was considered individually.

Histology.—Tissues were fixed in formalin, cut at 5 to 6 microns and stained with hematoxylin and eosin.

Hormone assay.—Fluids and extracts were tested for estrogenic hormone by the vaginal smear method of biological assay using doubly ovariectomized adult albino rats. Aqueous preparations were injected three times subcutaneously in eight hours and oil preparations once. Smears were read at 48, 54, 72 hours. Because the amounts of material available for testing were usually very limited, a strictly quantitative assay was impossible. However, graded doses were used wherever possible. A "rat unit" has been arbitrarily defined as the smallest amount of fluid tested which gave a positive estrous response.

Extracts.—When extraction was desired the following method was employed: Two volumes of 95 per cent alcohol were added to 1 volume of fluid and the mixture was allowed to stand. The precipitate was removed by centrifugation and the supernatant fluid filtered through a Jena filter. The precipitate was washed twice with 95 per cent alcohol and once with anhydrous ether. The combined supernatant fluid and washings were acidified to Congo red with HCl, and evaporated dropwise under reduced pressure on a water bath. The residue was triturated 4 times with 25 cc. portions of anhydrous ether and the extract was filtered through a Jena filter. The ether solution was added dropwise to a measured amount of olive oil and the ether removed. A separate extraction was made for each amount tested. Extractions were made in triplicate when fluid was available.

Control studies.—For control studies fluids from nonovarian cystic adnexal tumors and ascitic fluids have been assayed for estrogenic hormone. These tumors consist of parovarian cysts, cystic fibroids, etc.

Classification and Incidence

In general, classifications of ovarian tumors are unsatisfactory. For the purpose of this investigation a simple classification based upon morphologic findings has been used without attempts at further refinement. The classification and the summary of the data are shown in Table I. Ovarian cysts have been classified either as benign or malignant. The benign tumors have been subdivided into 1. single type tumors in which only one type of cyst was found, although sometimes these cysts were multilocular in nature; 2. multiple type tumors in which more than one type of cyst was present; 3. bilateral cysts. The single type group is comprised of follicle and corpus luteum cysts, representing pseudoneoplasms, and simple serous, papillary serous, pseudomucinous, dermoid and a miscellaneous group, consisting of cysts devoid of epithelial lining, degenerate forms and otherwise unclassified types. The series is essentially complete except for dermoids which are often solid or semisolid and have not always been tested for hormone.

The series consists of 193 cases of ovarian cysts; 172 were benign and 21 malignant. Excluding the dermoids the series consists of 168 benign ovarian cysts of which 134 or 79.8 per cent were single type. In the benign group 17 or 10.1 per cent of the patients gave histories of previous operations for ovarian cysts or other ovarian disease and are classed as "recurrent" cysts; 15 or 8.9 per cent had multiple tumors; 19 or 11.3 per cent had bilateral involvements; 3 of which were also multiple in type. Of the 21 cases of ovarian carcinoma there was one case of bilateral tumors. In the single type group the incidence of various types of cysts excluding dermoids was: follicle 11.9 per cent, corpus luteum 11.9 per cent, simple serous 35.8 per cent, papillary serous 11.2 per cent, pseudomucinous 21.6 per cent, miscellaneous 7.5 per cent.

Table I: Classification and Summary—Cystic Adnexal Tumors

	Cases		Tumors		Fluids	
	No.	Total	No.	Total	No.	Total
Benign ovarian cysts		172		189		294
Single type	138		138		211	
*Follicle	16		16		18	
*Corpus luteum	16		16		17	
Simple serous	48		48		68	
Papillary serous	15		15		32	
Pseudomucinous	29		29		61	
Dermoid	4		4		5	
Miscellaneous	10		10		10	
Multiple type	15		15		41	
Bilateral	19		36		42	
Malignant ovarian cysts		21		23		55
Benign nonovarian cysts		26		27		28
Embryonic rests	13		14		15	
Miscellaneous	8		8		8	
Endometriosis	5		5		5	
Totals		219		239		377
Ascitic fluids (assoc. with tumors)		26				36

* Pseudoneoplasms.

Twenty-seven cystic adnexal tumors were included for control studies. They include 13 tumors arising from embryonic rests (6 parovarian, 2 bilateral parovarian, 3 wolffian, 1 hydatid of Morgagni, 1 Gartner's duct), fluid from 5 cases of endometriosis, 3 cystic fibroids, 4 hydrosalpinx, 1 Bartholin cyst. Thirty-six ascitic fluids were also examined.

Age Incidence

Since the ovary is characterized by a very rapid succession of tissue and hormonal changes over the

long period during which it is functionally active, it is of interest to consider the occurrence of ovarian cysts during various decades of life. Fig. 1 shows the age incidence for 155 benign and 19 malignant ovarian cysts of this series. The data include all the benign cysts except those of patients having a history of a previous cystectomy or oophorectomy and in which the present tumor is considered as the second occurrence of a tumor. The 3 decades, 15 to 45 years of age, have been considered to represent the period of the greatest functional activity of the ovary. Seventy-three per cent of the benign tumors were removed during this period and 26 per cent after this age. In the malignant tumor group, 58 per cent were removed after 45 years of age. The group of malignant tumors is very small. Although the number of cysts in each group of the single type cysts is too small to be significant, there seems to be a tendency for different types of cysts to occur at different ages.

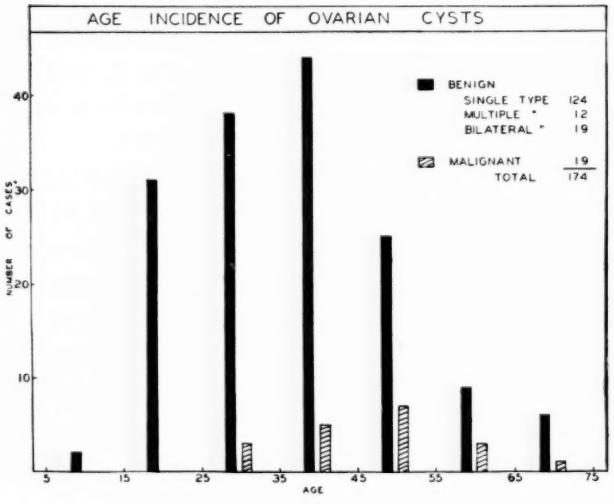

FIG. 1

OCCURRENCE OF ESTROGENIC HORMONE IN SINGLE TYPE OVARIAN CYSTS AT VARIOUS AGES

In order to avoid confusion because of the presence of different types of cysts in the same tumor or patient only single type cysts have been analyzed for estrogenic hormone during the various age periods. In the group shown in Fig. 2-A representing 124 cases 35 per cent of the cysts between ages 15 to 45 show estrogenic hormone. These data include both follicle and corpus luteum cysts in which hormone could be expected to occur. If these cysts are excluded so that only the true neoplasms, 96 cases, are included the data appear as shown in Fig. 2-B. In the age period 15 to 45 years, 21 per cent show estrogenic hormone. Thirty-six per cent of all the positive cysts were found in the patients over 45 years of age, at a period when the ovary is presumably quiescent. In the two decades after 55, 5 of 10 cysts showed hormone.

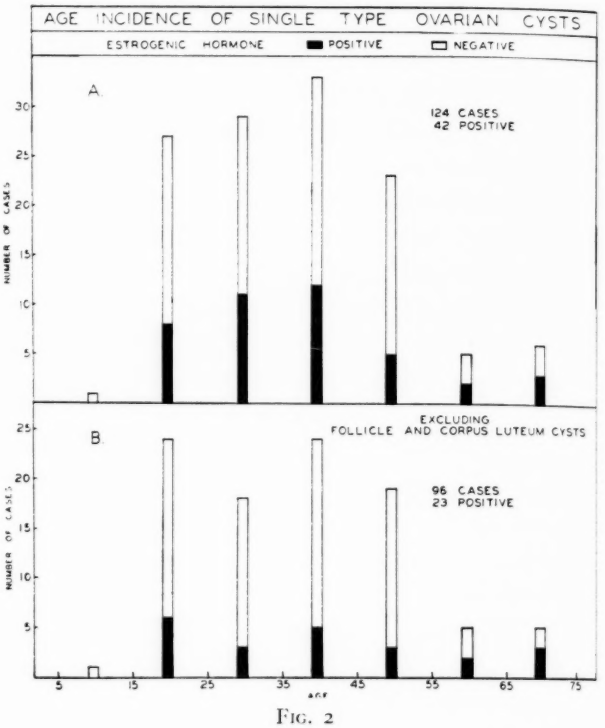

FIG. 2

OCCURRENCE OF ESTROGENIC HORMONE IN SINGLE TYPE OVARIAN CYSTS

The data in single type cysts represented in Fig. 2-A have been analyzed according to the type of cysts which show estrogenic hormone. This analysis appears in Fig. 3 and shows the percentage of cysts with positive fluids as follows: follicle 86.7 per cent, corpus luteum 46.2 per cent, simple serous 26.8 per cent, papillary serous 21.4 per cent, pseudomucinous 17.9 per cent, dermoids none, miscellaneous 33.3 per cent.

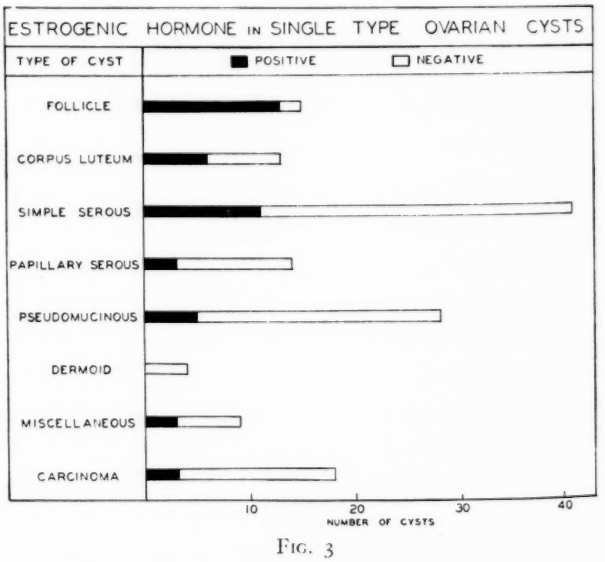

FIG. 3

Estrogenic Hormone in Fluids of Cystic Adnexal Tumors

General.—Assays for estrogenic hormone have been made on 377 fluids from 239 cystic adnexal tumors and 36 fluids associated with tumors, usually malignant. These data appear in Table II. The ovarian cyst fluids have been classified according to the type of lining of the cyst. The combined data for single and multiple type, and bilateral tumors and the "recurrent" ovarian cysts comprise 294 fluids from 189 benign tumors, and 55 fluids from 23 malignant tumors.

The amount of fluid tested depended upon the quantity of fluid available and upon the type of cyst. The amount of fluid obtained has been used as a measure of the size of the cyst; the actual size of the cyst or tumor is sometimes much greater than this especially when "cystic ovaries" or corpus luteum cysts are concerned. When the type of cyst was uncertain and the amount of fluid was too limited for testing graded doses, usually ½ to ⅓ the total material was injected so that a negative response indicated less than 2 to 3 rat units per cyst. Fluids were not tested at higher levels than 200 cc. per dose because it was thought that body fluids and tissues in general might contain a comparable amount of hormone. When characteristically negative fluids were tested at low levels it usually indicated small amounts of material.

The percentage of positive fluids for the different types was: follicle, 61.8; corpus luteum, 48.4; simple serous, 24.1; papillary serous, 12.2; pseudomucinous, 12.0; dermoids, none; miscellaneous, 38.5; malignant, 5.5 (Table II).

Table II: Occurrence of Estrogenic Hormone in Ovarian Cyst Fluids

	Single type			All other types									Total		
				Multiple		Bilateral		Recurrent		Total					
Type of cyst	No. of fluids	No. positive	Per cent positive	No. of fluids	No. positive	No. of fluids	No. positive	No. of fluids	No. positive	No. of fluids	No. positive	Per cent positive	No. of fluids	No. positive	Per cent positive
Follicle	17	13	76.5	5	2	7	3	5	3	17	8	47.1	34	21	61.8
Corpus luteum	13	6	46.2	2	2	10	3	6	4	18	9	50.0	31	15	48.4
Simple serous	58	12	20.7	6	0	8	3	11	5	25	8	32.0	83	20	24.1
Papillary serous	30	3	10.0	6	1	3	0	2	1	11	2	18.2	41	5	12.2
Pseudomucinous	60	5	8.3	7	2	12	0	4	3	23	5	21.7	83	10	12.0
Dermoid	5	0	0	1	0	2	0	1	0	4	0	0	9	0	0
Miscellaneous	9	3	33.3	3	1	0	0	1	1	4	2	50.0	13	5	38.5
Total	192	42	21.9	30	8	42	9	30	17	102	34	33.3	294	76	25.9
Carcinoma	38	3	7.9	0	0	9	0	8	0	17	0	0	55	3	5.5

Relation of Occurrence of Estrogenic Hormone to the Amount of Fluid Tested

The amount of fluid tested depended not only upon the type of cyst but also upon the amount of fluid available. The amounts actually tested are shown on a greatly compressed scale in Fig. 4. Each dot and each circle represents the final assay value for a different fluid. The positive fluids are shown by dots at the values of the smallest amounts of fluid tested which gave positive responses, the circles indicate the largest amounts of fluid tested which still gave negative responses. Amounts of fluid tested ranged from 0.03 cc. to 200 cc. Fluids of different types showed positive responses in characteristic ranges but not all fluids of any type contain hormone. The amount of fluid required to give a positive response ranged from 0.03 cc. in some follicle fluids to more than 200 cc. in some pseudomucinous fluids. Because the amounts of fluid were often small, certain fluids could not be tested in a range where positive tests might be anticipated.

Concentration of Estrogenic Hormone in Ovarian Cyst Fluids

When estrogenic hormone is found in ovarian cyst fluids the concentration seems to be characteristic for different types of fluid. Fig. 5 shows the concentration per cc. expressed on a greatly compressed scale. For the positive fluids dots represent rat units per cc. and for the negative fluids circles indicate the presence of less than that number of rat units. Follicle fluids range from 0.07 to 33 rat units per cc., corpus luteum from 0.12 to 2, simple serous 0.005 to 0.2, papillary serous

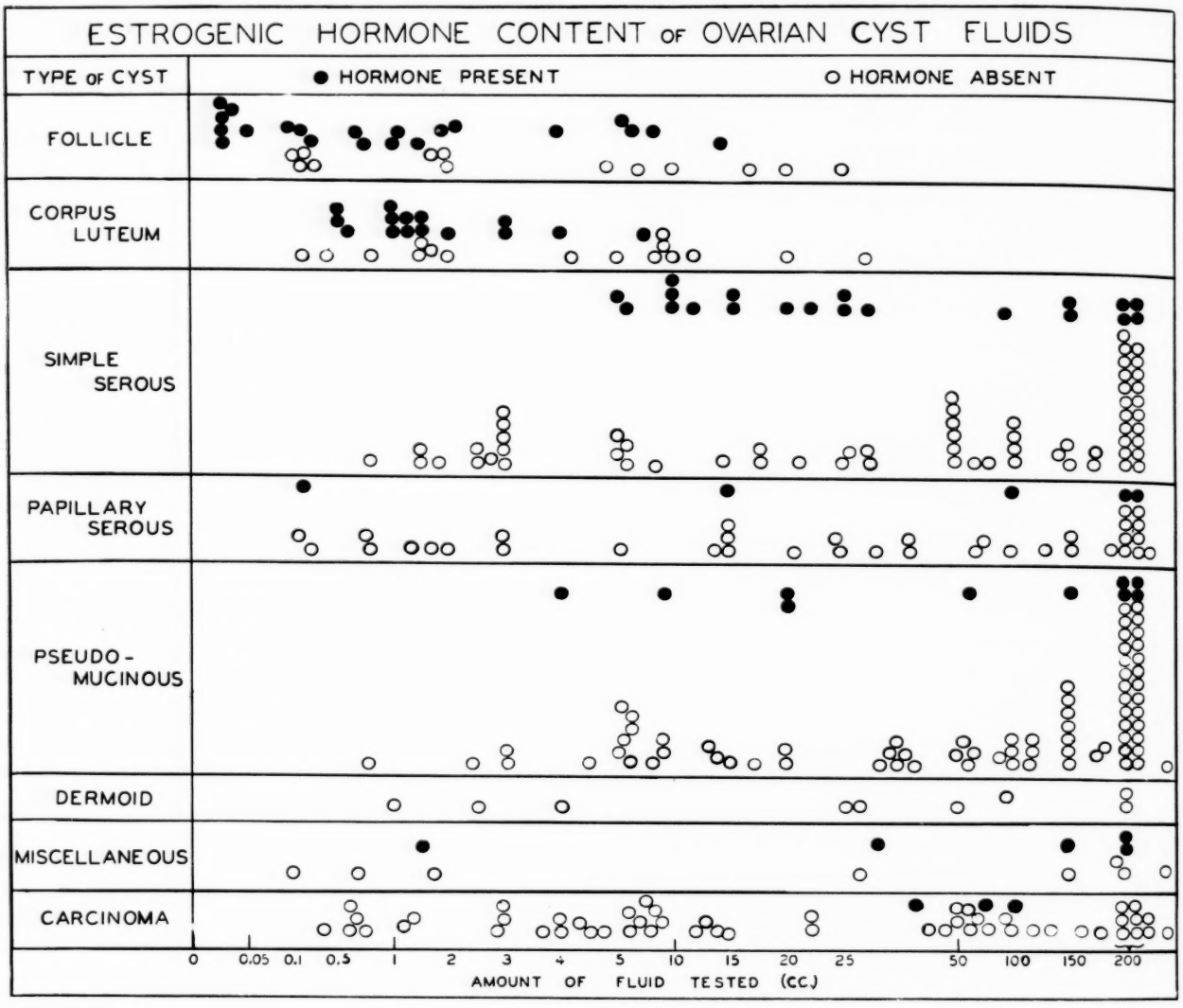

FIG. 4

and pseudomucinous showed scattered values in the same range but were characteristically negative, dermoids were negative, 3 malignant cysts showed 0.01 to 0.03 rat units per cc.

THE TOTAL AMOUNT OF ESTROGENIC HORMONE IN OVARIAN CYSTS

As previously shown (1) the size of the cyst probably is not a determining factor in regard to the presence or absence of estrogenic hormone. The amount of fluid obtained has been used as a measure of the size of the cyst. Cyst fluids ranged in amount up to 20 liters. Hormone was found in large as well as in small cysts. Apparently its absence in large cysts is not a result of dilution as the cyst increases in size. The absence of hormone in small cysts indicates that diffusion into the cyst from ovarian structures and storage are probably not important factors.

In single type cysts the total amount of estrogenic hormone per cyst, expressed in rat units, was 1.3 to 215 for follicle, 1.7 to 96 for corpus luteum, 1.4 to 225 for simple serous, 7.5 to 200 for papillary serous, 1.1 to 16 for pseudomucinous cysts. The total amount of hormone found in the cysts was not characteristic for any type of cyst, but seemed to depend not only upon the amount of hormone per cc. of fluid but also upon the size of the cyst.

ESTROGENIC HORMONE IN FLUIDS OF SINGLE TYPE CYSTS

The occurrence of estrogenic hormone in fluids of single type cysts differs from that of the entire group of fluids. This difference is due to a different distribution of positive fluids in the multiple and bilateral groups and in recurrent cysts derived from patients with histories of previous ovarian disease. The group

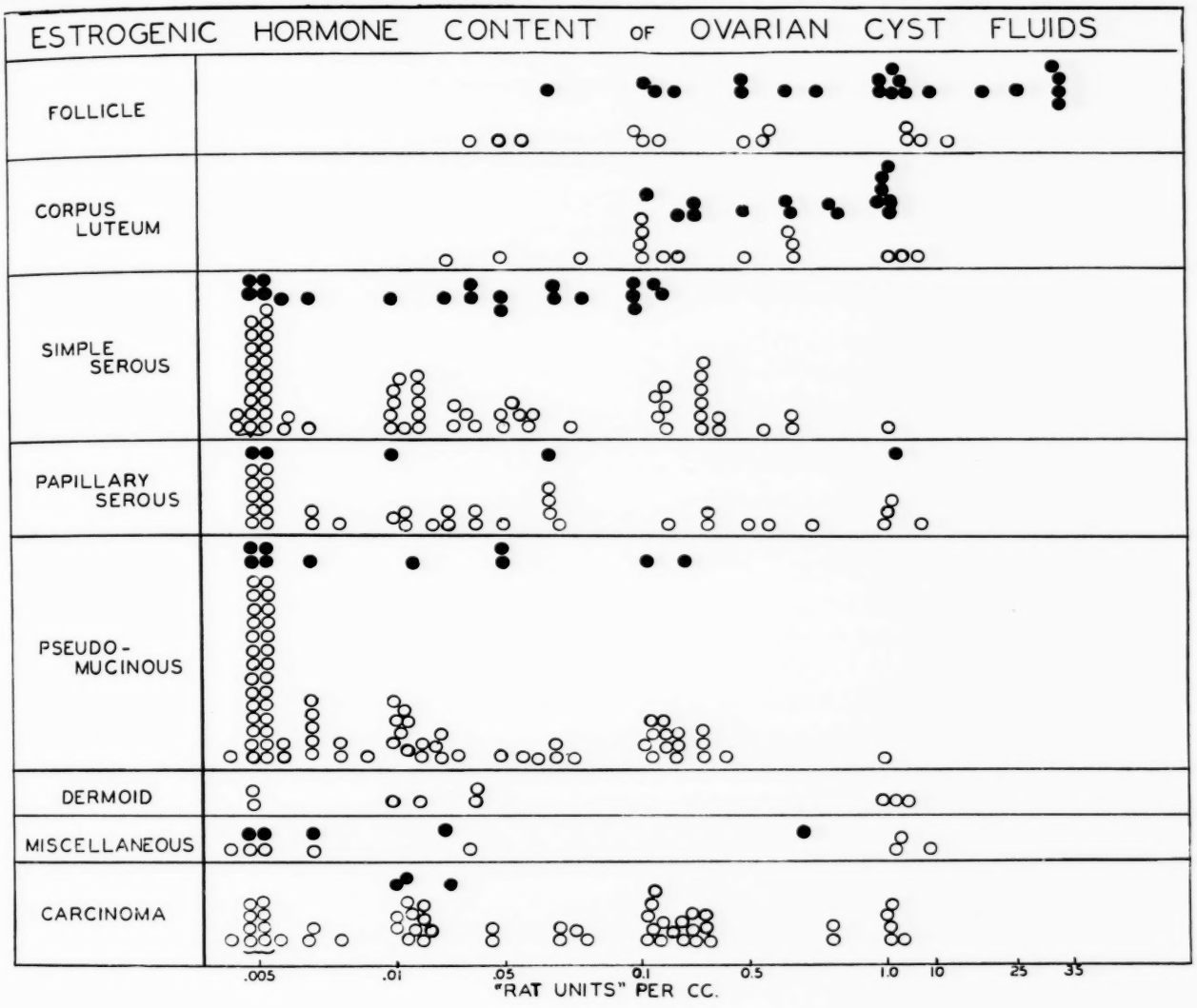

FIG. 5

of cases is the same as that in Fig. 3 but represents the total number of fluids instead of tumors. The data appear in Table II and Fig. 6. The percentages of positive fluids are: follicle 76.5 per cent; corpus luteum 46.2 per cent; simple serous 20.7 per cent; papillary serous 10.0 per cent; pseudomucinous 8.3 per cent; dermoid none; miscellaneous 33.3 per cent. The differences in the two groups are shown, for example, in the incidence of 76.5 per cent positive follicle fluids in single type fluids in contrast to 47.1 per cent in other groups combined and 8.3 per cent positive single type pseudomucinous fluids with 21.7 per cent in the other groups. These differences will be discussed later.

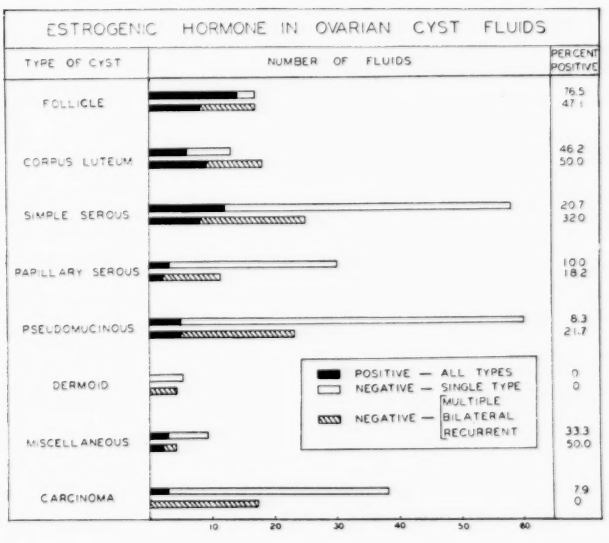

FIG. 6

MULTIPLE TYPE OVARIAN CYSTS

The multiple type group of ovarian cysts includes those tumors which contain more than one type of cyst. Fifteen tumors of this type have been examined

and the data appear in Table II and III. Three other tumors of this type also appear in the group of bilateral tumors (Table IV). Multiple type tumors are various combinations of cystic structures, both neoplastic and pseudoneoplastic. Three of the patients gave histories of previous ovarian disease. Two of these cysts and three others involved primarily the follicle or corpus luteum. Pseudomucinous cysts also occurred commonly in these tumors and were the chief cyst in 3 tumors and the minor cyst in 2. Simple serous cysts were the major cyst in 2 and minor in 3. Papillary serous cysts occurred as major in 3 and minor in 2. Five dermoid cysts were found in this group. Dermoid, pseudomucinous and follicle cysts have been found in one tumor. Apparently cysts can grow adjacently without diffusion of hormone from one cyst fluid to the other. Two of the 3 cysts which were primarily pseudomucinous in type showed hormone and 1 of these was a "recurrent" cyst. One of the 3 papillary serous contained hormone.

Bilateral Ovarian Cysts

The group of bilateral ovarian cysts includes 19 cases in which both ovaries showed cysts or ovarian disease and were removed simultaneously. When tumors or ovaries have been removed at some previous time the present tumors have been classed in a "recurrent" group and are shown in Table V. Of the 19 cases in this group, 3 were multiple type tumors. Of the 16 cases of single type cysts, 6 showed the same type of cyst on the two sides—1 follicle, 1 lutein, 1 simple serous, 1 papillary serous, 2 pseudomucinous; 2 of the bilateral multiple type showed bilateral dermoids and the other bilateral cysts with combined simple serous and corpus luteum cysts. Another showed a follicle cyst on one side and 2 corpus luteum cysts in the other ovary. Seven patients showed follicle or corpus luteum cysts on one side and serous, pseudomucinous, or dermoid on the other. Two were combined pseudomucinous and simple serous. Ten of the 16 cases showed either follicle or corpus luteum cysts in one ovary. The occurrence of bilateral tumors of different types (other than follicle or corpus luteum cysts) is of interest. The positive fluids were found among the follicle and corpus luteum cysts with the exception of 2 cases of bilateral simple serous cysts in which 3 of 4 cysts contained estrogenic hormone.

Recurrent Ovarian Cysts

This group includes 17 patients with histories of previous ovarian cystectomies or oophorectomies performed at other institutions. The data appear in Tables II and V. Three of the tumors were also multiple type. In the remaining 14 cysts the incidence was: 1 follicle, 3 corpus luteum, 7 simple serous, 1 papillary serous, 1 pseudomucinous, 1 with no epithelial lining. The large percentage of positive fluids from the true neoplasms is to be noted. Excluding the follicle and corpus luteum cysts, 10 of the 19 (53 per cent) fluids of this group contained estrogenic hormone in contrast to 14.2 per cent in the same group of single type cysts. One patient in the general series with a large simple serous cyst with fluid which contained estrogenic hormone developed a malignant tumor a year later. There are three cases of recurrent tumors in the group of malignant ovarian tumors.

Malignant Ovarian Cysts

There are twenty cases of unilateral and one of bilateral malignant ovarian cysts in this series. Three patients had been operated upon previously; one for a cyst and two for ovarian carcinoma. These three tumors were probably of a papillary serous type and were negative or contained less than 1.1, 1.5, 4.6 r. u. per cyst. Only 3 of the 55 fluids tested were positive for estrogenic hormone, 2 of these were from papillary serous cysts. Considering the types of fluid examined the amounts tested were often too small to permit anticipation of finding the hormone. Of the two papillary serous cysts one contained 0.03 r. u. per cc. and 63 r. u. per cyst and the other 0.01 r. u. per cc. and 3.5 r. u. per cyst. The third cyst contained 0.013 r. u. per cc. and 24 r. u. per cyst. The per cent in which hormone was found corresponds with that of the benign series. It may be noted that not all parts of the same tumor present evidence of malignancy or the same degree of malignancy.

Nonovarian Cystic Adnexal Tumors

Twenty-seven nonovarian cysts have been tested for estrogenic hormone in order to determine its occurrence in extra-ovarian fluids. Fourteen of these were cysts of embryonic origin and included 6 cases of unilateral parovarian cysts, 2 bilateral parovarian, 3 wolffian, 1 hydatid of Morgagni, 1 Gartner's duct. With one exception, all of these fluids were negative; the positive fluid was from a parovarian cyst and contained 0.02 r. u. per cc. and 10.6 r. u. for the entire cyst. Fluids were negative at 200 cc. in 4 cases. Fluids from 4 cases of hydrosalpinx, 3 cystic fibroids and 1 Bartholin cyst were all negative. Five fluids associated with endometriosis were also negative.

Ascitic Fluids

Ascitic fluid from 26 patients with tumors has been tested for estrogenic hormone. Twenty of the 36 fluids were tested at 200 cc.; usually extractions and tests were made in triplicate. All of the 36 fluids were negative.

TABLE III: ESTROGENIC HORMONE IN MULTIPLE TYPE OVARIAN CYSTS

Number	Age	Type	Amount of fluid in cc.	Amount tested (cc.)		Estrogenic hormone, R.U. per cc.		Previous operation for ovarian disease
				Positive	Negative	Present	Less than	
11 A	32	Corpus luteum	12	...	5	...	0.2	Cystectomy
C		Follicle	10	1.0	...	1.0	...	.....
B		Follicle	1.5	1.3	...	0.77	...	.....
281 A	40	Follicle	14.5	0.75	...	1.3	...	None
C		Papillary serous	1.5	...	0.8	...	1.3	.....
B		No epith. lining	0.8	...	0.7	...	1.4	.....
154	28	Corpus luteum (old)	38	...	30	...	0.03	Cystectomy
A		Simple serous	7	...	3	...	0.33	.....
190 B	27	Corpus luteum	23	1.0	...	1.0	...	None
A		No epith. lining	13	1.5	...	0.67	...	.....
C		No epith. lining	0.12	...	0.12	...	8.3	.....
296 A	34	Corpus luteum	90	7.5	...	0.13	...	None
C		Follicle	0.3	...	0.2	...	5.0	.....
D		Follicle	0.25	...	0.2	...	5.0	.....
B		Simple serous	2	...	1.8	...	0.56	.....
143	39	Simple serous	145	...	50	...	0.02	None
A		Aspirated †	3.5 *	...	2	...	0.5	.....
152	35	Simple serous	607	...	200	...	0.005	None
A		Aspirated †	0.6 *	0.05	...	20	...	.....
110	44	Papillary serous	1,235	...	200	...	0.005	None
A		Papillary serous	218	...	100	...	0.01	.....
B		Follicle	2	...	2	...	0.5	.....
118	57	Papillary serous	10,260	200	...	0.005	...	None
A		Pseudomucinous	96	...	90	...	0.012	.....
B		Pseudomucinous	32	...	30	...	0.033	.....
151	43	Papillary serous	1,850	...	200	...	0.005	None
A		Pseudomucinous	440	...	200	...	0.005	.....
54	63	Pseudomucinous	480 ‡	...	100	...	0.01	None
A		Aspirated †	28	...	10	...	0.1	.....
101	29	Pseudomucinous	1,660	20	...	0.05	...	Oophorectomy
A		Pseudomucinous	25	20	...	0.05	...	.....
B		Pseudomucinous	9.5	9	...	0.11	...	.....
D		Follicle	39	...	20	...	0.05	.....
E		Follicle	46	...	25	...	0.04	.....
C		Dermoid	28.6	...	28	...	0.036	.....
158	40	Pseudomucinous	3,200	200	...	0.005	...	None
A		Pseudomucinous	23	...	20	...	0.05	.....
B		Pseudomucinous	75	60	...	0.017	...	.....
C		Papillary serous	17	...	15	...	0.067	.....
139 D	19	Dermoid §	..	...	...	...	...	None
139		Simple serous	202	...	100	...	0.01	.....
A		Simple serous	31	...	29	...	0.034	.....
B		Simple serous	16	...	14	...	0.071	.....
C		Aspirated †	52	...	45	...	0.022	.....
234 A	28	Dermoid	148	...	50	...	0.02	None
B		Follicle	2.8	0.04	...	25	...	.....

* Composite.
† Aspirated and no histology.
‡ Incomplete specimen.
§ Not tested.
See Table IV.

TABLE IV: ESTROGENIC HORMONE IN BILATERAL OVARIAN CYSTS

Cyst No. 1				Estrogenic hormone				Cyst No. 2			Estrogenic hormone			
				Amount tested (cc.)		R.U. per cc.					Amount tested (cc.)		R.U. per cc.	
Number	Age	Type	Amount of fluid in cc.	Positive	Negative	Present	Less than	Number	Type	Amount of fluid in cc.	Positive	Negative	Present	Less than
67	31	Follicle	19	...	7	...	0.14	68 A	Follicle	12.5	4	...	0.25	....
								68 B	Follicle *	0.47	...	0.2	...	5.0
10 A	27	Corpus luteum	9	4	...	0.25	....	10 B	Corpus luteum	4	...	1.5	...	0.67
247 A	38	Papillary serous	222	...	190	...	0.005	248	Papillary serous	77	...	65	...	0.015
B		Papillary serous	0.6	...	0.3	...	3.3	...	.....	...	...	...	...	....
268	37	Simple serous	165	25	...	0.04	....	269	Simple serous	101.5	90	...	0.01	....
74	62	Pseudomucinous	3,800 †	...	200	...	0.005	75	Pseudomucinous	90 †	...	50	...	0.02
208	21	Pseudomucinous	175	...	150	...	0.007	209 A	Pseudomucinous	7	...	6	...	0.17
								C	Pseudomucinous	142	...	120	...	0.008
								D	Pseudomucinous	43	...	35	...	0.028
236	48	Simple serous	1,703	...	200	...	0.005	237	Follicle	2	...	1.7	...	0.59
166 A	39	Corpus luteum	27	1.5	...	0.67	....	165	Follicle	5.8	0.03	...	33.3	....
B		Corpus luteum	20	...	8.8	...	0.11	...	.....	...	...	...	...	....
45	26	Pseudomucinous	5,360	...	200	...	0.005	46	Follicle	26	2.1	...	0.5	....
72	14	Pseudomucinous	192	...	115	...	0.009	71	Follicle	35	...	10	...	0.1
297	43	Pseudomucinous	212	...	180	...	0.006	298	Follicle	2.2	...	1.6	...	0.6
141	22	Pseudomucinous	2,260	...	200	...	0.005	142	Follicle *	8	0.05	...	20	....
30	48	Pseudomucinous	3,350	...	150	...	0.007	31	Simple serous	210	...	140	...	0.007
39	58	Simple serous	2,200	...	200	...	0.005	40	Pseudomucinous	2,000	...	150	...	0.007
229	44	Dermoid	218	...	200	...	0.005	230	Corpus luteum	3.5	...	2	...	0.5
303	23	Simple serous	120	...	80	...	0.013	304	Corpus luteum	10	3	...	0.33	....
260 A	33	Dermoid	109.5	...	95	...	0.01	261	Dermoid ‡	...	...	...	...	....
B		Corpus luteum	0.3	...	0.2	...	5	...	.....	...	...	...	...	....
270	26	Simple serous	9.0	...	5.0	...	0.2	272 B	Simple serous	31	22	...	0.05	....
B		Corpus luteum	1.0	...	0.4	...	2.5	A	Corpus luteum	12	...	9.25	...	0.11
282	31	Dermoid ‡	...	...	...	...	....	...	.....	...	...	...	...	....
A		Corpus luteum	6.6	...	4.05	...	0.25	...	Dermoid ‡	...	...	...	...	.

* No histology. † Incomplete specimen. ‡ Not tested.

TABLE V: ESTROGENIC HORMONE IN RECURRENT OVARIAN CYSTS

Number	Age	Single type	Amount of fluid in cc.	Amount tested in cc.		Estrogenic hormone R.U. per cc.		Previous operation for ovarian disease
				Positive	Negative	Present	Less than	
96	36	Follicle	7.6	2.0	...	0.5	...	Oophorectomy
245 A	41	Corpus luteum	13.5	3.0	...	0.33	...	Oophorectomy
B		Corpus luteum	13.0	0.6	...	1.67	...	.....
262	25	Corpus luteum	69	1.2	...	0.83	...	Ooph., resection
289	40	Corpus luteum	30	1.0	...	1.0	...	Oophorectomy
18	49	Simple serous	37	...	18.0	....	0.06	Oophorectomy
66 A	39	Simple serous	260	15	...	0.07	...	Oophorectomy
B		Simple serous	120	15	...	0.07	...	.....
98	34	Simple serous	3,990	200	...	0.005	...	Oophorectomy
130	56	Simple serous	52	...	20.0	....	0.05	Oophorectomy
A		Simple serous	14	...	3.0	....	0.33	.....
B		Simple serous	8	...	3.0	....	0.33	.....
146	40	Simple serous	955	...	200	....	0.005	Oophorectomy
156	24	Simple serous	815	150	...	0.007	...	Oophorectomy
184	36	Simple serous	362	150	...	0.007	...	Cystectomy
134	46	Papillary serous	89	15	...	0.067	...	Oophorectomy
A		Papillary serous	44	...	25	....	0.04	.....
138	67	Pseudomucinous	9,000	...	200	....	0.005	Cystectomy
256	37	No epith. lining	234	200	...	0.005	...	Resection
		Multiple type						
11 A	32	Corpus luteum	12	...	5	....	0.2	Cystectomy
B		Follicle	1.5	1.3	...	0.77	...	.....
C		Follicle	10	1.0	...	1.0	...	.....
101	29	Pseudomucinous	1,660	20	...	0.05	...	Oophorectomy
A		Pseudomucinous	25	20	...	0.05	...	.....
B		Pseudomucinous	9.5	9	...	0.11	...	.....
C		Dermoid	28.6	...	28	....	0.036	.....
D		Follicle	39	...	20	....	0.05	.....
E		Follicle	46	...	25	....	0.04	.....
154	28	Corpus luteum (old)	38	...	30	....	0.03	Cystectomy, oophorectomy
A		Simple serous	7	...	3	....	0.33	.....

SUMMARY

In an attempt to correlate the occurrence of estrogenic hormone in cystic ovarian tumors with the morphology of the tumor 212 ovarian cysts have been examined. Of these 189 were benign and 23 were malignant. As a control study 27 cystic nonovarian genital tumors and 36 ascitic fluids associated with tumors have been studied.

Seventy-three per cent of the benign ovarian cysts were removed between the ages of 15 to 45 years, during the period of the greatest functional activity of the ovary. Fifty-eight per cent of the malignant ovarian cysts were removed after 45 years of age. Estrogenic hormone was found in 35 per cent of the benign cysts in the age group 15 to 45 years. If those cysts arising from known ovarian structures (follicle and corpus luteum) are excluded, 23 per cent of the cysts contain hormone. In this group of neoplastic cysts 28 per cent of the cysts from patients over 45 years of age contain hormone. This comprises 36 per cent of all the positive cysts in this group.

The percentage of different types of single type cysts containing estrogenic hormone were: follicle, 86.7; corpus luteum, 46.2; simple serous, 26.8; papillary serous, 21.4; pseudomucinous, 17.9; dermoids, none; miscellaneous, 33.3. Considering all the fluids of these cysts, which include multilocular cysts, the following percentages of positive fluids were obtained: follicle, 76.5; corpus luteum, 46.2; simple serous, 20.7; papillary serous, 10.0; pseudomucinous, 8.3; dermoids, none, miscellaneous, 33.3. Fluids from multiple type and bilateral cysts and cysts from patients with histories of previous ovarian disease (recurrent group) showed a different percentage incidence of positive fluids, as follows: follicle, 47.1; corpus luteum, 50.0; simple serous, 32.0; papillary serous, 18.2; pseudomucinous,

21.7; dermoid, none; miscellaneous, 50.0. These differences result from (a) fewer positive follicle fluids in all the groups but especially in the multiple and bilateral groups; (b) a larger number of positive simple serous fluids in the bilateral and recurrent groups; (c) a higher per cent of positive pseudomucinous fluids in the multiple and recurrent groups. Excluding follicle and corpus luteum fluids, 10 of 17 of the positive fluids in the special group are found in the recurrent group and 3 of 17 in the bilateral group.

When estrogenic hormone was present in ovarian cyst fluids the amount found varied with the type of cyst. The concentration per cc. of fluid, expressed in rat units, was as follows: follicle, 0.07 to 33; corpus luteum, 0.12 to 2; simple serous, 0.005 to 0.2; papillary serous and pseudomucinous showed only a few positive fluids in scattered range; dermoids were negative.

In single type cysts the total amount of estrogenic hormone per cyst depended upon the concentration of the hormone and the amount of cyst fluid. The total amounts, in rat units, for various types of fluids were: follicle, 1.3 to 215; corpus luteum, 1.7 to 96; simple serous, 1.4 to 225; papillary serous, 7.5 to 200; pseudomucinous, 1.1 to 16.

Twenty-three malignant ovarian cysts have been studied. Only 3 of the 55 fluids tested contained estrogenic hormone. One contained 0.03 rat units per cc. and a total of 63 rat units, another 0.01 rat units per cc. and a total of 3.5 rat units and the third 0.013 rat units per cc. and 24 rat units per cyst.

Fourteen cysts arising from embryonic rests were tested and with the exception of one parovarian cyst all were negative for estrogenic hormone. Fluids from 4 specimens of hydrosalpinx, 5 from endometriosis, 3 cystic fibroids, 1 Bartholin cyst, and 36 ascitic fluids were also negative for hormone.

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Some Cytologic Effects of Therapeutic Radiation

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In a series of experiments an attempt has been made to determine the effect of different types and dosages of radiation on cytoplasmic components at different intervals after irradiation. This is a report of a quantitative study of the centrioles in interkinetic cells of the Walker rat carcinoma 256 following radiation (single dose 2,400 r and 4,800 r) and of the mouse sarcoma CR 180 (single dose 2,400 r). Also quantitative observations were made on the grossly abnormal mitoses in the radiated tissue.

Review of Literature

It has been shown that with appropriate technic centrioles can be demonstrated in Walker rat carcinoma 256. Also there appears a variation in the frequency of multiple centrioles (more than the normal two) following a single dose of 2,400 r. In addition, irregularly shaped nuclei, giant cells, multipolar mitoses, and other evidences of grossly abnormal cell divisions were observed by Fogg and Warren (6).

Many workers have reported on the effect of radiation on nuclei, mitoses, and the formation of abnormal cells. Recently, Dederer (2) reported the production of giant spermatocytes in *Philosamia cynthia* after x-ray dosages of 2,000 r to 8,000 r. She notes that the giant cells may be of different types, single, binucleate, or multinucleate and concludes these variations must have been produced by the fusion of cells rather than by nuclear division. Whitman (12) radiated normal fibroblasts in tissue culture and Walker rat carcinoma 338 and noted abnormalities, especially in the nucleus. Kemp and Juul (7) confirmed the earlier work of Donaldson and Canti (3) and of Strangeways and Hopwood (11) which showed that abnormalities could be produced by radiation but that these did not differ essentially from cells growing in an unfavorable medium. Crowther (1), in a theoretical review on the biological action of x-rays, states as his belief that the destructive nonspecific action of radiation is due to chemical changes in the cytoplasm. Packard (9) has reviewed the literature and recently a general discussion of the subject with bibliographies has been presented in Duggar's *Biological Effects of Radiation* (4).

Observations

The method of killing the animals, the nature of the rat carcinoma 256, and the preparation of the tissue were the same as described in previous papers (5, 6). By using identical technic it was possible to show bodies usually paired and probably centrioles in the mouse sarcoma CR 180. At intervals of 18, 24, 48, 72, 96, and 120 hours after radiation animals were killed and the tumor tissue prepared for study. Tumors from a nonradiated rat and a nonradiated mouse were also fixed and stained to serve as controls. The paired bodies embedded in a surrounding mass of acidophilic centrosomal substance which are identified as centrioles can be seen in Fig. 1.

Fig. 1.—Nonradiated interkinetic cell of the Walker rat carcinoma 256 showing 2 centrioles in relatively clear acidophilic area near nucleus. Photograph of Bouin preparation, phosphotungstic acid hematoxylin stain. Mag. × 3,500.

The observations on the rat tumor after a single dose of 2,400 r are summarized in Table I. It will be noted that 18 hours after radiation 10 per cent of the interkinetic cells in which centrioles were to be seen show more than 2 centrioles as contrasted with 4 per cent in the nonradiated control. The greater number of these multiple centriole cells have 3 or 4 centrioles. Actually it was often difficult to determine whether the number was 3 or 4.

By 24 hours 15 per cent of the counted cells show multiple centrioles. There are a few which have more than 4 centrioles. Table III shows that approximately 94 per cent of the mitoses at this interval present some apparent gross abnormality, usually fragmented pieces of chromosomes or doubling of chromosomal substance in one cell.

We have reported (6) that with this tumor and dose the increase in the number of cells showing higher numbers of centrioles continues up to 72 hours. After 72 hours the percentage decreases although cells with a high number of centrioles persist. Note in Table I that there are 37 cells out of a thousand that contain 8 or more centrioles. It is worthy of note (Table III) that up to 72 hours there was also a high percentage of cells exhibiting abnormal mitoses. The abnormal mitoses at 48 hours to 72 hours and later showed multipolar mitoses, widely scattered chromosomes, and the occurrence of giant cells in profusion. Subsequent to 72 hours the number of abnormal mitoses decreases and by 120 hours following radiation it is approaching the control condition.

The effect of a single dose of 4,800 r on the same tissue is recorded in Tables II and IV. An examination of Table II shows the same pattern of response as after 2,400 r; namely, an increase in the percentage of cells showing multiple centrioles up to about 72 hours and the appearance of cells having 6 or more centrioles. Then follows a gradual decrease. It is noteworthy that with the higher dose the percentage of cells showing multiple centrioles is not as high as with the 2,400 r. However, Table IV shows that while high percentages of abnormal mitoses continue longer than after 2,400 r the percentage remains about the same to 96 hours after which there is a lag of 24 hours before the drop toward the normal condition is apparent.

Tables V and VI show the effect of a single dose of 2,400 r on the mouse tumor CR 180. The high frequency of abnormal mitoses indicates a marked effect. This dose is very near the maximal for this tumor, as with greater doses almost complete necrosis occurred and the animal died. Our data after 72 hours show evidences of this lethal effect. At the 120-hour interval there was no significant number of cells in mitosis although giant cells and cells with fragmented nuclei showing many centrioles were abundant.

TABLE I: EFFECT OF 2,400 R ON CENTRIOLES IN WALKER RAT CARCINOMA 256

Hours after radiation	Number of cells counted	Number of centrioles per interkinetic cell									Per cent of multiple centrioles
		2	3	4	5	6	7	8	9	10	
18	1,000	900	81	17	1	0	1	0	0	0	10
24	1,000	848	40	100	2	10	0	0	0	0	15
48	1,000	605	97	274	5	15	2	2	0	0	40
72	1,000	401	62	355	14	113	7	41	1	6	60
96	1,000	538	77	236	11	94	7	30	0	7	46
120	1,000	966	26	6	2	0	0	0	0	0	3
Control	1,000	958	39	2	1	0	0	0	0	0	4

TABLE II: EFFECT OF 4,800 R ON CENTRIOLES IN WALKER RAT CARCINOMA 256

Hours after radiation	Number of cells counted	Number of centrioles per interkinetic cell									Per cent of multiple centrioles
		2	3	4	5	6	7	8	10	12	
18	500	480	9	11	0	0	0	0	0	0	4.0
24	500	484	3	9	0	4	0	0	0	0	3.2
48	500	442	14	40	0	3	0	0	1	0	12.0
72	500	437	7	43	0	11	0	1	0	0	12.6
96	500	443	3	50	0	3	0	1	0	0	11.4
120	500	461	1	27	0	2	0	5	2	2	7.8
Control	1,000	958	39	2	1	0	0	0	0	0	4.0

TABLE III: EFFECT OF 2,400 R ON MITOSES IN WALKER RAT CARCINOMA 256

Hours after radiation	Number of cells counted	Normal mitoses	Gross aberrations	Per cent of aberrations
1-18	No significant number of mitoses, normal or abnormal			
24	500	28	472	94.4
48	500	42	458	91.6
72	500	45	455	91.0
96	500	227	273	54.6
120	500	450	50	10.0
Control	500	489	11	2.2

TABLE IV: EFFECT OF 4,800 R ON MITOSES IN WALKER RAT CARCINOMA 256

Hours after radiation	Number of cells counted	Normal mitoses	Gross aberrations	Per cent of aberrations
1-18	No significant number of mitoses, normal or abnormal			
18	500	68	432	86.4
24	500	50	450	90.0
48	500	42	458	91.6
72	500	51	449	89.8
96	500	53	447	89.4
120	500	248	252	50.4
Control	500	489	11	2.2

However, at the earlier intervals the consistency of the response to the radiation is apparent although the percentage of cells showing multiple centrioles was less for each interval than was noted in Walker carcinoma 256 after similar treatment. On the other hand both tissues consistently showed a very high percentage of abnormal mitotic divisions.

It will be further noted (Table VII) that the percentage of abnormal mitoses in all three tissues studied is approximately the same through the 96-hour interval. In contrast, the percentage of multiple centrioles in Walker carcinoma 256 following a single dose of 2,400 r is consistently higher than in the same tissue following a single 4,800 r dose or in the mouse sarcoma CR 180 following a single 2,400 r dose.

Discussion

Every slide of radiated tissue studied showed necrotic cells, varying in number, and it is suggested that as a result of radiation most of the cells suffer injury, lethal in some, transient in others. The hypothesis has previously been advanced that the temporary increase in the number of centrioles is due to the fact that cytoplasmic division of certain cells is inhibited although nuclear division and the division of the centriole continue within limits. The preponderance of centrioles in multiples of two further supports this suggestion. Also the number of centrioles per cell does not reach its maximum until a lapse of time adequate for the production of several cell generations. The possibility of the formation of centromeres or fragmentation of the centriole bears further investigation.

The data suggest that a single dose of 4,800 r approaches the maximal single dose for the rat as 2,400 r does for the mouse. One may deduce from this that the vitality of the cells is so reduced by the radiation that few of the cells which initiate division are capable of performing subsequent abortive divisions which might give rise to multiple centrioles. Whereas, in Walker carcinoma 256 treated with 2,400 r, the lesser injury permits a greater number of cells to continue through later abortive divisions thus producing a greater number of multicentriolar cells. The data suggest further that if a cell initiates division before 120 hours the mitosis will usually be abnormal. If a radiated cell delays division until after 120 hours the effect of the radiation has been reduced to such a point that the process will probably be normal.

Table V: Effect of 2,400 r on Centrioles in Mouse Sarcoma CR 180

Hours after radiation	Number of cells counted	Number of centrioles per interkinetic cell									Per cent of multiple centrioles
		2	3	4	5	6	7	8	10	12	
18	311	300	3	8	0	0	0	0	0	0	3.5
24	691	635	10	40	0	5	0	1	0	0	8.1
48	495	354	9	72	1	42	0	13	4	0	28.8
72	500	250	6	87	1	63	0	59	27	7	50.0
96	353	184	2	68	0	27	0	36	24	12	47.8
120	221	37	1	78	0	44	0	33	14	14	83.2
Control	515	500	3	12	0	0	0	0	0	0	3.0

Table VI: Effect of 2,400 r on Mitoses in Mouse Sarcoma CR 180

Hours after radiation	Number of cells counted	Normal mitoses	Gross aberrations	Per cent of aberrations
1-18	No significant number of mitoses, normal or abnormal			
18	500	43	457	91.4
24	500	50	450	90.0
48	500	42	458	91.6
72	500	35	465	93.0
96	500	49	451	90.2
120	No normal mitoses			
Control	500	473	27	5.4

Table VII: Summary of Effects of Different Dosages of Radiation on Tissue

Hours after radiation	Per cent of multiple centrioles			Per cent of abnormal mitoses		
	2,400 r 256 *	4,800 r 256 *	2,400 r CR 180 †	2,400 r 256 *	4,800 r 256 *	2,400 r CR 180 †
18	10	4.0	3.5	...	86.4	91.4
24	15	3.2	8.1	94.4	90.0	90.0
48	40	12.0	28.8	91.6	91.6	91.6
72	60	12.6	50.0	91.0	89.8	93.0
96	46	11.4	47.8	54.6	89.4	90.2
120	3	7.8	83.2	10.0	50.4	...
Control	4	2.9	3.0	2.2	2.2	5.4

* Walker carcinoma 256.
† Mouse sarcoma CR 180.

Summary

Centrioles can be demonstrated in the Walker rat carcinoma 256 and the mouse sarcoma CR 180 after suitable fixation and staining.

Radiation causes an increase in the percentage of cells in both tissues showing multiple centrioles for a period of 24 to 96 hours.

In Walker carcinoma 256 with doses of 2,400 r and 4,800 r it was the higher dose which gave a lower percentage of multiple centrioles for any given period after radiation.

A quantitative study of multiple centrioles shows that sarcoma CR 180 with a dose of 2,400 r is more sensitive to radiation than Walker carcinoma 256 after a similar dose.

From the time mitosis is resumed after irradiation the percentage of cells showing abnormal mitoses is consistently high for a period up to 96 hours for both tumors regardless of dose.

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Further Studies on the Effect of X-Rays on a Tumor of Known Genetic Constitution

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In two previous reports (6, 8) attention was called to an apparent transmissible, genetic change induced in the transplantable mouse tumors (dbrB and Simpson) by comparatively small amounts of x-rays. The change from a specific to a nonspecific tumor following x-radiation manifested itself by the fact that radiated tumors were successfully propagated in several pure strains of mice in which the nonradiated tumor fails to grow. For example, these tumors will not grow in the strains of mice known as the A stock albinos, Little's C57 black and Strong's CBA. On the other hand, when these tumors, either radiated or nonradiated, were transplanted into their host strains there were always approximately 100 per cent takes. However, when exposed *in vivo* in the host strains to doses of 50 to 100 r (λ effective 0.16 A.), and transplanted one week following the radiation into the above mentioned strains, these tumors grew in approximately 40 per cent of the animals. Furthermore the radiated tumors have been successfully propagated through several transplantations in the resistant strains of mice and then successfully transplanted back into the original host strain, indicating that the change induced in the tumors by the radiation was a permanent one for at least 5 transplant generations.

The studies presented in this paper deal with the effect of varying doses of x-radiation on the percentage takes of the Simpson tumor in the C57 black strain. The doses of radiation (λ effective 0.16 A.) varied from 25 to 1,000 r. The same experimental procedure was followed as was described in the previous papers (6, 8). The percentage takes in the so-called resistant strain, C57 black, increased with increased dosage up to a certain point after which there was a decrease. These experimental data are compiled in Table I and have also been plotted on arith log paper as shown in Fig. 1, with the magnitude of the percentage probable errors shown by the vertical lines for each point. As this curve is drawn there is an exponential relation between the dose and the percentage takes. For doses of 0 to 25 r the exponent is 2.26 while for doses of 25 to 400 r the exponent is 1.01.

Muller (5) working with mature male germ cells of *Drosophila* has determined that while the frequency of gene mutations bears a simple linear relationship to the dose administered, the frequency of structural change is an exponential function of the dose, the exponent for doses of 1,000 to 4,000 r being about 1.5 whereas the exponent for lower doses is greater, approaching 2.0, the square of the dose.

Our exponents are comparable to those of Muller in that a large exponent is associated with small doses and a small exponent with larger doses. The explanation for this break in the curve is not at first apparent, but Muller gives a clue. He explains the gene mutations on the theory of individual successful ionizations, and the structural changes on the basis of multiple breaks and translocations of the chromosomes as a result of direct hits. In the latter case, he claims that the recombination is blocked in the spermatozooan stage until fertilization occurs, at which time the fragments are subject to more movement as a result of cell division. This process of cell division favors new combinations of the broken chromosomes.

TABLE I: RELATION BETWEEN PERCENTAGE TAKES AND DOSAGE

Dose r	Total no. animals	No. animals positive	Percentage positive	Probable error (percentage)
25	35	12	34.4	± 5.4
50	24	8	33.4	± 6.5
100	90	41	45.5	± 1.12
200	56	27	48.2	± 4.5
250	26	14	54.0	± 6.6
400	30	21	70.0	± 5.6
600	41	25	61.0	± 5.5
800	17	12	70.6	± 7.5
1000	15	8	53.4	± 8.7

When considering somatic cells of the Simpson tumor from this point of view, there can be no block such as is mentioned by Muller in connection with the germ cells. However, if cell division is more favorable to new combinations of broken chromosomes and the doses of radiation administered to the tumor cells did result in direct hits which split the chromosomes, then it is possible to theorize that low doses, having apparently little or no effect on cell division, afford a maximum opportunity for new combinations of the fragments. To go one step further, it is generally

accepted that increasing doses of radiation have an increasing effect of retardation on cell division. In fact it has been shown by Love (4) and Gordon (3) that doses of the order of 100 to 400 r are capable of markedly reducing mitotic activity. It might be expected, therefore, that if the mitotic activity of the

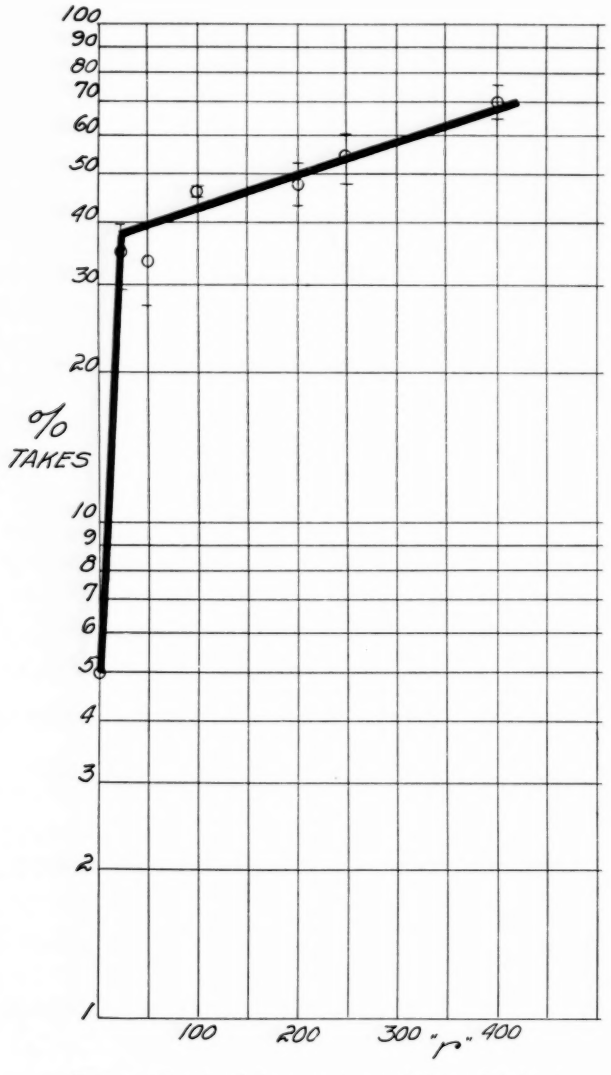

FIG. 1.—Graph of the experimental data of Table I for doses from 0 to 400 r. As drawn this is an exponential relation between the percentage takes and dosage for a dose 0 to 25 r and another exponential relation for doses of 25 to 400 r.

radiated cells is decreased, the possibility of new combinations of the chromosomes is reduced following radiation with doses from 25 to 400 r. Thus the lowered probability of new combinations would cause the exponent to be smaller with increased dosage.

If the theory is correct that progressively increasing doses tend progressively to decrease mitotic activity, in addition to the direct action on the chromosomes, we would have to rule out constant exponents for the two portions of the curve for doses up to 400 r. Instead there should be a progressively decreasing exponent. In other words, our graph should not be represented by straight lines but by a smooth curve. The portion of the curve up to a dose of about 25 r may represent the threshold dose for effect on mitotic activity. Our experimental points could then be connected as shown in Fig. 2. Additional experimental work is in progress to check this curve.

The question whether or not we have produced a genetic change in these tumors by means of radiation is not necessarily answered by the work presented so far, although the fact that these tumors, in addition to growing in the resistant strains, have been carried through several transplant generations, would seem

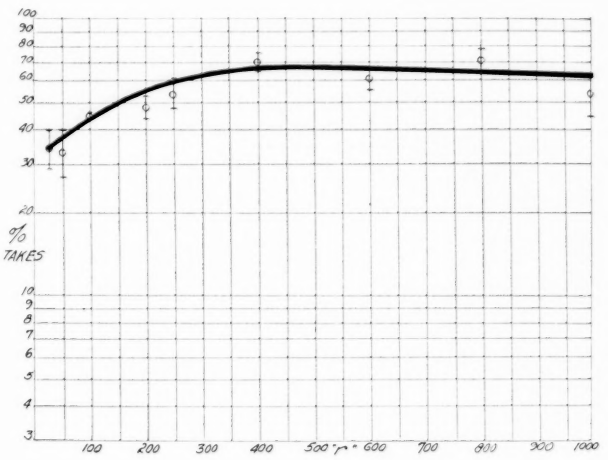

FIG. 2.—Graph of the experimental data from Table I for doses from 25 to 1,000 r, where the experimental points are connected with a continuous curved line which represents a progressively decreasing exponent.

to bear out the contention that we have produced a permanent genetic change.

According to Bittner (1), a genetic change is always in the direction of more malignancy and less tissue specificity. Our radiated tumors are definitely less specific. An indication of a genetic change, according to Schultz (7), is the absence of any time factor and as is further pointed out by Demerec (2), there is no recovery in the case of hereditary effects—such changes, once they have occurred, do not revert.

In order to determine the influence of the time factor and recovery effect, we have carried out the following experiment.

Tumors of the Simpson strain were radiated *in vivo* with 200 r and transplanted into one of the so-called resistant strains of mice (94 animals of C57 black strain), according to the following schedule: immediately after radiation, after 1 day, 3 days, 5 days, and

7 days. The results of this experiment to date show that the percentage takes, approximately 40 per cent, hold fairly constant for all the time intervals mentioned, indicating that the effect is instantaneous and does not depend on any latent period such as is normally associated with radiation experiments. Furthermore, although the number of animals used for this work to date is not sufficient for conclusive proof, there is no evidence of a recovery from the radiation.

Conclusions

Based on the experimental work performed to date on the Simpson tumor, we believe that:

1. An inheritable genetic change has been produced by radiation doses as low as 25 r.

2. The percentage takes with varying doses is intimately connected with the effect of the radiation on cellular activity as well as the effect on the genetic constitution.

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Genetics of Melanomas in Fishes

V. The Reappearance of Ancestral Micromelanophores in Offspring of Parents Lacking These Cells*

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(Received for publication May 29, 1941)

In previous papers in this series (5, 8, 13) it has been shown that spontaneous malignant melanomas are produced in the hybrid offspring of the black-spotted platyfish, *Platypoecilus maculatus,* and the swordtail, *Xiphophorus hellerii.* The melanomas are evoked genetically in hybrids by the interaction of the sex-linked, heritable factor for macromelanophores, *Sp,* of the platyfish with a series of *Sp* modifiers, *A* and *B,* of the swordtail. These melanomas may be said to be produced experimentally because the platyfish and the swordtail probably do not crossbreed under natural conditions in Mexico. The author (10) has collected nearly 10,000 specimens of these two species in their native habitat without finding a single natural hybrid, despite the fact that both species were often found living side by side.

Two Kinds of Melanophores

In the fishes studied here, an important distinction has been drawn between a relatively small melanophore, 0.3 mm. in size, and a considerably larger one which may attain a size of 1.2 mm. at its widest dimension. The smaller, micromelanophores, are found normally in the dermal areas of the integument and in the tissues that surround the large blood vessels and the spinal cord; they are found in the meninges of the brain, in the lining of peritoneum, and in the retina. The larger, macromelanophores, in normal fish, are restricted in their distribution, being found only in scattered areas of the corium.

The micro- and macromelanophores differ not only in their distribution and in morphology but also in the method of inheritance, the micromelanophore factor, *St,* being autosomal while the macromelanophore factor, *Sp,* is sex-linked.

The most striking distinction between these two melanophores is made evident in the development of melanomas in the hybrids of the black-spotted *Platypoecilus maculatus* with other species. Melanomas have appeared repeatedly in a Mendelian proportion of hybrid broods, but they appear only in those fish which are genetically dominant for macromelanophores, *Sp.* Micromelanophores, *St,* in the absence of macromelanophores, are incapable of evoking the melanomas. However, the presence of micromelanophores as well as of the increased numbers of macromelanophores, *Sp St,* intensify the melanotic tumor in the hybrid fish. Further details of the part played by each type of melanophore are given by the author (5, 8).

Melanomas with Known Types of Melanophores

For the purpose of the tissue culture study (14), it had been planned to use melanomas from fish hybrids that had only one type of corial melanophore, the macromelanophores. The author (8) pointed out that melanomatous fish of this kind could be developed by mating a spotted platyfish (a *Platypoecilus maculatus* variety, possessing macromelanophores, *Sp,* but no micromelanophores, *st*) with a golden swordtail (a *Xiphophorus hellerii* variety lacking both types of melanophore, *sp st*). It was believed that the interpretation of the cell types in tissue cultures could be clarified by the use of material having only one kind of melanophore.

At the time when plans were completed for the culture of fish tissue, melanotic hybrids of a spotted platyfish with the golden swordtail were not available.

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* Through the courtesy of Dr. Robert Chambers of New York University an opportunity was presented for the study of fish melanomas by the tissue culture technic. Cultures were made of the melanomas that were obtained from the hybrids of a black-spotted platyfish and an albino swordtail. These are described in the paper that follows (14).

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‡ The author wishes to thank Dr. Charles M. Breder, Jr., director, and the New York Zoological Society, managers, of the New York Aquarium for the extensive facilities provided for the breeding and rearing of the experimental animals at their institution.

Instead, we had highly melanotic hybrids between the spotted platyfish and the albino swordtail [1] (Fig. 1).

At first it had seemed to us that the substitution of an albino for the golden swordtail would not alter the melanophore complex of the melanotic hybrid since neither the albino nor the golden swordtail possesses micro- or macromelanophores;—at least, they do not show them phenotypically. We now have evidence, however, that the albino swordtail is genetically dominant for micromelanophores, *StSt,* but it does not show these pigment cells in the presence of a homozygous recessive inhibitor of melanin pigment, *ii*.

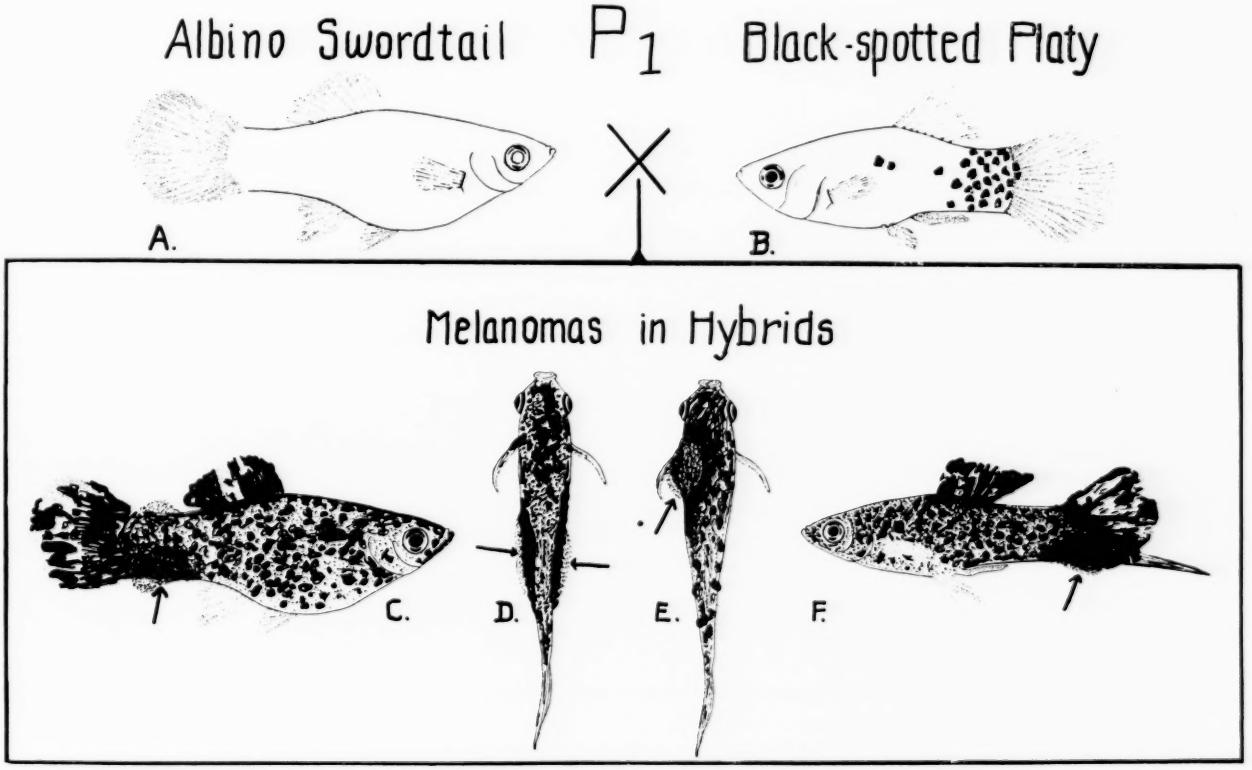

FIG. 1.—Origin of melanomas in hybrid fishes. When an albino swordtail, without black pigment (upper left), is mated with a macromelanophore-carrying platyfish (upper right) their hybrid offspring (lower line) give rise to melanomas. Neither the albino swordtail nor the spotted platyfish parents show any micromelanophores; nevertheless their melanomatous offspring (F_1) have these small pigment cells in addition to the macromelanophores. This is demonstrated by the genetical analysis in the text.

Since it is difficult to distinguish between micromelanophores and macromelanophores under conditions of extreme melanosis, the evidence for the presence of micromelanophores in the spotted platyfish × albino swordtail hybrid has been determined by a genetic method of analysis.

The author (6, 7) has shown that when the wild type, olive-green swordtail (*StSt*) is mated with the aquarium-developed, black-eyed, golden mutant (*ststst*) the wild type is dominant. This conclusion has been reached independently by Kosswig (18) who employs the term *f* for the golden and *F* for the wild type. Simple Mendelian inheritance was also found by Kosswig (17) when he crossed the albino swordtail (*ii*) with the dominant wild type (II). Our unpublished data confirm Kosswig's results on this point. Kosswig, in 1935, employed the term *pp* for the albino and *PP* for the wild type, but since, in 1931, Gordon and Fraser used the symbols *P* and *p* in their earlier study of a dominant series of allels in *Platypoecilus* for entirely different characters, *I* and *i* have been substituted in this paper for *P* and *p*.

When the albino swordtail (*ii*) is mated to a golden swordtail (*ststst*), the author (9) has found that the original color of the *wild* swordtail, with its full complement of micromelanophores, is restored as follows:

Albino swordtail × Golden swordtail = Wild swordtail
No micromel. × No micromel. = Micromelanophores.
ii StSt × *II ststst* = *Ii Ststst*.

When *Ii Ststst* swordtails are mated *inter se,* these ratios appear, approximately, in F_2: 9 wild type, 3 golden and 4 albinos.[2]

The genetic constitution of the fish bearing melanomas used in the present tissue culture studies may therefore be expressed in the following manner:

Albino swordtail × Black-spotted platyfish = Melanotic hybrid.
ii StSt spsp AA BB × *II ststst SpSp aa bb* = *Ii Ststst Spsp Aa Bb*.

[1] These were kindly provided by Mr. Fred Flathman, an aquarist of Woodhaven, Long Island, New York; duplicate hybrids were reared at the New York Aquarium.

[2] Unpublished data.

I represents normal coloring, the wild gray; *i* represents a recessive inhibitor of melanin pigment development; in a homozygous state *ii* denotes albinism.

St represents the dominant factor for micromelanophores, the wild gray color; *stst,* when in a homozygous recessive state, represents an animal that is golden, black-eyed. However, *StSt ii* denotes albinism.

Sp indicates the presence of macromelanophores; *sp* in the homozygous state represents lack of macromelanophores.

A and *B* represent multiple factors of the swordtail which act in conjunction with *Sp* for macromelanophores of the platyfish to change the growth habits of these melanophores from the normal to the pathological. Further details concerning the multiple effects of *A* and *B* may be found in Gordon (8).

The first use of the albino factor in the production of melanomas in fish hybrids was made by Breider (1, 2). His results were later discussed by Kosswig (18). Breider (1) reported the mating of a black-banded platyfish to a swordtail. The black female hybrid was backcrossed to an albino swordtail and then a black offspring was backcrossed again to an albino. Later, Breider (2) mated a black-spotted red hybrid to an albino swordtail. All these melanotic hybrids were derived from platyfish parents that had both micro- and macromelanophores. Thus the genetical analysis, outlined above, could not be made from their experiments alone.

The great influence of *i* in the homozygous state upon the phenotypic expression of black spots, *Sp,* and of black band, *N,* both of which contain macromelanophores, and of the wild pattern, *St* (micromelanophores) is explained by Breider and by Kosswig. They also discuss the role of *i* in the formation of colorless tumors.

Similarity of Melanomas

The melanotic tumors in the spotted platyfish × albino swordtail hybrids of the F_2 (Fig. 2) are histologically similar to other spotted platyfish-swordtail hybrids described by Reed and Gordon (19), Haussler (15), Smith, Coates, and Strong (20), and Gordon and Smith (12). They are essentially similar, also, to melanomas found in hybrids between the spotted platyfish, *P. maculatus,* and the other species of *Platypoecilus: couchianus, xiphidium,* and *variatus* described by Gordon and Smith (13). In every case on record involving a platyfish hybrid combination, melanomas have always been associated with the presence of macromelanophores derived from *P. maculatus.*

Other types of pigment-bearing cells may occasionally be seen in platyfish × swordtail hybrid melanomas such as xanthophores and xantho-erythrophores. They have their origin in either the platyfish or swordtail parent or both according to Goodrich, Arrick, and Hill (3). Even the albino swordtail has these two types of chromatophores in a much reduced number. They seem to take no essential part in the formation of the neoplasm. In a rare case Kosswig (16) found an erythrophoroma, but the genetic basis of this tumor was not established.

Summary

Melanomas in hybrids of the platyfish and the swordtail are said to be experimentally produced because these two species have never been found to crossbreed in their native habitat in Mexico.

The fish melanoma used in tissue culture studies, described by Grand, Gordon, and Cameron (14) were obtained by mating a spotted platyfish with an albino swordtail.

In terms of melanophore content, the platyfish has macromelanophores only. These cells are inherited and are referred to a sex-linked, dominant factor, *Sp.*

The albino swordtail is without a trace of melanophores, small or large; that is, they do not show the melanophores phenotypically. It has been found, however, that the albino swordtail, genotypically, like the wild type, is dominant for micromelanophore. These cells are inherited through an autosomal factor, *St.* The albino swordtail does not show its micromelanophores because of the presence of a homozygous recessive factor, *ii,* an inhibitor of melanin. The albinos used in these experiments have the genetic constitution *StSt ii.*

The hybrid offspring of a spotted platyfish (macromelanophores only) and an albino swordtail (no visible melanophores) have two kinds of melanophores: a) macromelanophores and b) micromelanophores. This combination is brought about by the interaction of the factors *Sp, St,* and *I* all of which are represented in the melanomatous hybrids of the first generation.

The melanomas are produced by the interaction, specifically, of the factor for macromelanophores, *Sp,* of *Platypoecilus maculatus* with the *Sp* modifying factors *A* and *B* of *Xiphophorus hellerii.*

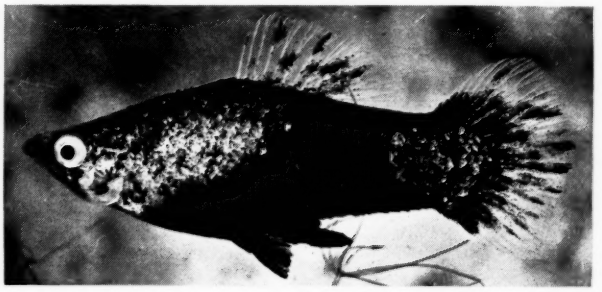

Fig. 2.—Melanoma in hybrid fish. The lower half of the mid region of the body bears a melanotic tumor, although the entire integument is involved. A dorsal view of this specimen may be seen in Fig. 1 D. This individual and the others in Fig. 1 were the source material for tissue culture studies of fish melanomas (14).

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Neoplasm Studies VIII

Cell Types in Tissue Culture of Fish Melanotic Tumors Compared with Mammalian Melanomas*

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The fish melanomas used in these studies were obtained from the hybrid offspring of a black-spotted platyfish (*Platypoecilus maculatus*) and an albino swordtail (*Xiphophorus hellerii*), and from hybrids of a spotted *Platypoecilus maculatus* and *Platypoecilus couchianus*. The melanomas are evoked genetically in the former hybrids by the interaction of the macromelanophore factor, *Sp,* of the platyfish with the heritable macromelanophore-growth modifiers, *A* and *B,* of the swordtail. The genetic aspect of this problem is treated in a paper (3) which precedes this one.

The distribution of the macromelanophores in the body of the purebred platyfish is restricted to a few scattered areas of the corium. In the aforementioned hybrids, on the other hand, the growth of the macromelanophores is radically changed. Reed and Gordon (7) have shown that the hypertrophy of macromelanophores brings about, first, the complete replacement of the normal tissues of the corium and, second, the penetration into the external fascia that separates the corium and the muscles. The macromelanophores further invade the deeper regions by following the fascial tissues along the horizontal septum, the septa of the mid-dorsal, mid-ventral, and deeper muscle masses, and finally surround the individual muscle fibres. As development continues, the corium in various localized regions becomes increasingly thickened and the swellings, when viewed in cross section, were found to be due to the proliferation of relatively small, spindle-shaped, pigment-bearing cells which seem to be of a new type from that of the macromelanophore. These cells continue the invasion to the extent of replacing muscle fibres, periosteum of the vertebrae, precartilage and, in one specimen, they were found in the meninges of the spinal cord. Gordon and Smith (4) stated, from their study of cross sections, that the melanin-containing, spindle-shaped cells of the melanotic overgrowths in hybrid fishes histologically resembled the cells of mammalian melanosarcoma.

The purpose of applying tissue culture technic to the study of fish melanomas was to aid in the interpretation of the cellular elements involved and to compare the cell types with those described in cultures of mammalian melanomas by Grand, Chambers, and Cameron (6).

Technic

The melanomas are situated in the corium and occupy the region between the muscles and the epidermis. Because of the small size of the fish it was not easy to obtain sterile tissue for cultivation. The surface of the entire fish had to be disinfected for it was impossible to remove the epidermis which is only a few cells in depth. Numerous successive washings in sterile Ringer's solution did not prevent contamination. Hexylresorcinol was also found to be useless because a concentration sufficient to destroy the bacteria also inhibited the subsequent growth of the tissue. The best disinfectant proved to be a 1:10,000 solution of merthiolate made by diluting 1:1,000 merthiolate (Lilly) with sterile Holtfreter's solution.[1] The live fish was immersed in the merthiolate solution for 2 minutes and then transferred to a sterile Holtfreter solution before the tumor was aseptically removed. The excised tumor was placed in Holtfreter solution and cut into fragments approximately 0.5 mm. in size which were then mounted in a drop of a culture medium on cover slips, sealed over depression slides, and incubated at room temperature, 24-25° C.

Several culture media were used, the simplest being a drop of Holtfreter's solution in which a good growth of fibroblasts occurred in from 12 to 18 hours but in which no melanoblasts and very few macrophages were seen. The addition of chick embryo extract to Holtfreter's solution merely increased the extent of fibrocytic growth. Best results were obtained by using a medium composed of 1 drop of chicken plasma, 3 drops of fish serum, and 1 drop of chick embryo extract (in Holtfreter's solution). This mixture formed a soft coagulum in which all the cell types of the fish mela-

* This investigation was aided by a grant from The International Cancer Research Foundation. It was conducted in the laboratory of Dr. Robert Chambers to whom the authors express their deepest appreciation for advice and encouragement during the pursuit of these studies. This paper is a continuation of a series previously published in the *American Journal of Cancer*.

† Fellow, John Simon Guggenheim Memorial Foundation.

[1] Holtfreter's solution: NaCl, 0.35 gm.; $CaCl_2$, 0.01 gm.; KCl, 0.005 gm.; $NaHCO_3$, 0.02 gm.; distilled H_2O, 100 cc. This was sterilized by filtration through a Berkefeld N candle.

noma grew luxuriantly. The fish serum was obtained under sterile precautions from a carp or goldfish by inserting a 19 gauge hypodermic needle through the isthmus into the fish's heart. About 10 cc. of blood was obtained from a 25 cm. fish. The blood was expelled from the syringe into a sterile tube and centrifuged for 10 minutes at 2,000 r.p.m.; then the supernatant serum was pipetted off.

In this plasma serum extract medium, wandering macrophages and fibrocytes appeared in from 12 to 14 hours while melanoblasts and melanophores appeared in from 24 to 72 hours. Phenol red, as indicator, added to the culture medium, did not show the marked alkalinity observed for the Harding Passey mouse melanoma (6). Liquefaction of the medium, which usually occurs in cultures of mammalian malignant tissues, was evident also in cultures of fish melanoma. However, it was not found necessary to change the medium more often than once a week. With the usual care, and with weekly changes, cultures of fish tissues remained alive and active for many weeks.

Some difficulty was experienced in the fixation and staining of the cultures of fish tissues. On immersing the cover slips in the fixative, the clot frequently slipped off and curled up. This was partially prevented by gently dropping the fixative drop by drop onto the culture, or by burning the edges of the clot to the cover slip with a heated platinum needle before adding the fixative. In general, fish material did not stain as well with hematoxylin and eosin as mammalian and chick tissues. However, by using care, some good permanent preparations were obtained.

Observation on Cell Types in Fish Melanoma

The tissue cultures of material obtained in the early stages of melanosis formed scanty outgrowths while, in the latter stages of tumor development, the cultures grew luxuriantly.

Macrophages.—As in mammalian melanomas, the melanin-carrying macrophages were the first cells to migrate from the cultured fragments of fish tumor. This occurred within 12 to 14 hours of incubation. The migration of these cells was rather slow, doubtless because of the large amount of ingested melanin granules. Many macrophages were so heavily laden with large clumps of pigment that they appeared as round, black, almost inert bodies. Two are shown in Fig. 1. In cells slightly less burdened the melanin granules became clustered in that part of the cell opposite the direction of their movement. These macrophages gave the appearance of dragging behind them a mass of inert black pigment while their advancing pseudopodia contained practically no pigment. One of these melanin-laden macrophages is shown in Fig. 2. In still less pigmented macrophages the agglutinated melanin granules were distributed throughout the cytoplasm, including the multilaterally arranged pseudopodia.

The relative frequency of macrophages among other cell types in fish melanomas, such as the fibrocytes and melanoblasts, was not as great as in mammalian melanoma cultures. The macrophages retained their ingested pigment throughout life, releasing it only when the cells disintegrated.

Fibrocytes.—The fibrocytes appeared within 24 hours of the time of explantation, but always later than the macrophages. These spindle-shaped cells had oval nuclei and contained varying quantities of melanin pigment. In some fibrocytes the melanin granules were in clumped masses while in others they were dispersed around the nucleus. The pigment particles were rarely found in the extended cell processes.

On the whole, in cultures about 1 week old the fibrocytes, in relation to the other cells of the melanoma, were scanty in comparison to those in cultures of mammalian tissue. In later cultures the fibrocytes became more numerous (Fig. 4). A peculiar type of cell, which has been identified by Dederer (1) and Goodrich (2) as a "fan" or "canoe" cell, was found frequently among the fibrocytes. This cell, shown in Fig. 3, is spindle-shaped and has fine, delicate, outspread processes at two extremities. Some of these cells contained melanin granules; others did not.

Melanoblasts.—The first appearance of the melanoblasts was in 24-hour cultures. At this stage all that could be seen were their delicate, filamentous processes extending from the margin of the explant. In cultures of 72 hours or more the whole cell body of the melanoblast appeared beyond the explant. Fig. 4 shows a 3-week culture with an extensive outgrowth of melanoblasts intermingled with fibrocytes. High power views of two highly dendritic melanoblasts near the margin of the explant are shown in Figs. 5 and 6.

In the fish melanoma cultures the melanoblasts greatly outnumber the macrophages and fibrocytes. In some cultures they were almost the only cell type present.

Melanophores.—The term, melanophore, is used here to distinguish the relatively large melanin-carrying cells, characteristic of normal integument of fish, from the much smaller melanoblasts and the melanin-bearing macrophages and fibrocytes which appear only in the melanotic tumors.

Usually, melanophores remained within the explant but occasionally one or more appeared also in the medium. Within the explant, the highly pigmented processes of the melanophores remained in the expanded state, typical for fish melanophores, as shown in Figs. 7 and 8. In some cases the melanophores within the explant lost their extended processes, became

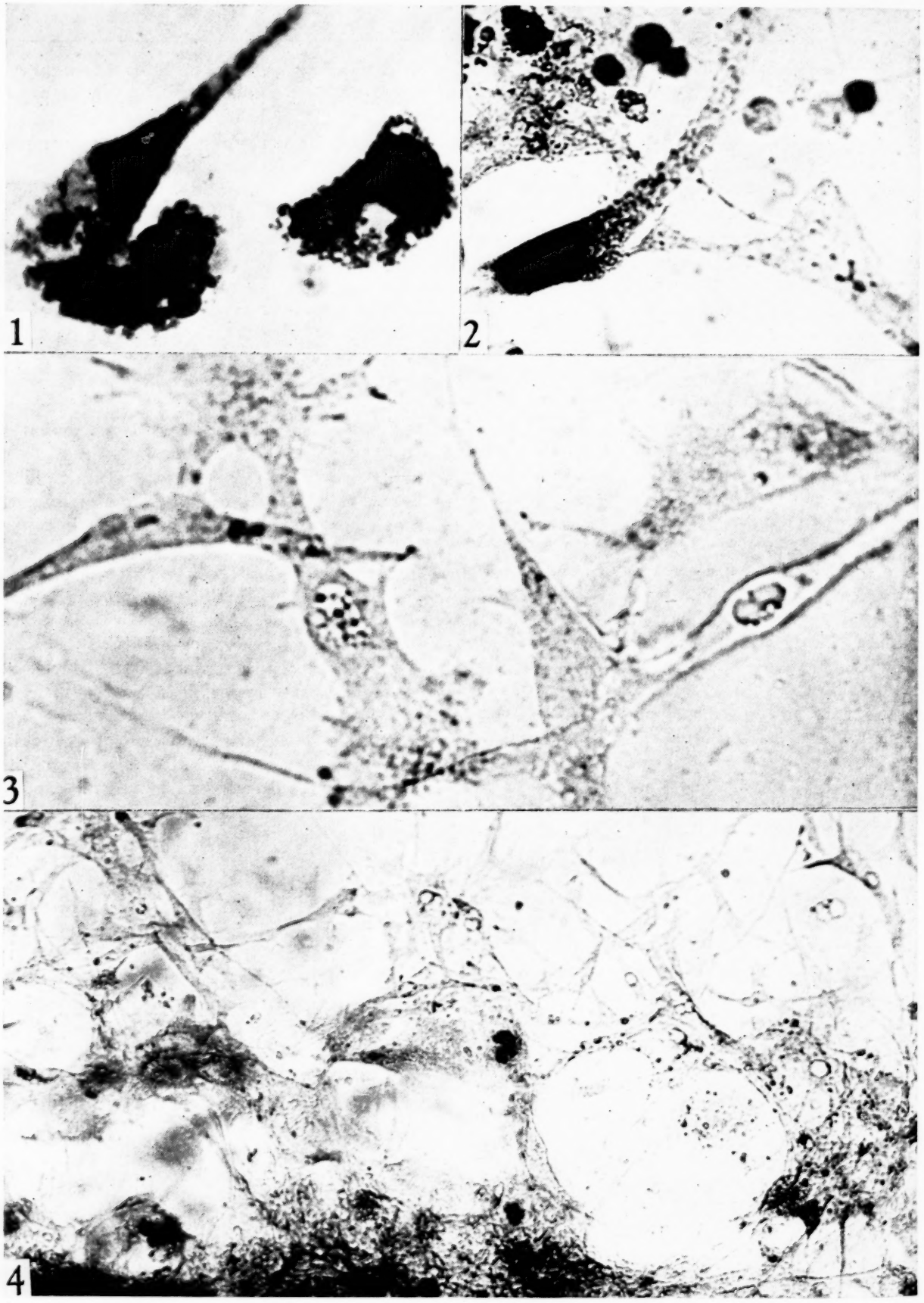

FIG. 1.—Two living macrophages heavily laden with melanin granules.

FIG. 2.—A living macrophage with accumulation of pigment at the posterior end. The advancing pseudopodia contain few pigment granules.

FIG. 3.—A group of living fibrocytes with one "fan cell." In some of the fibrocytes a few melanin granules were visible.

FIG. 4.—A low power view of a living 6-weeks-old culture of a rich outgrowth of melanoblasts and fibrocytes.

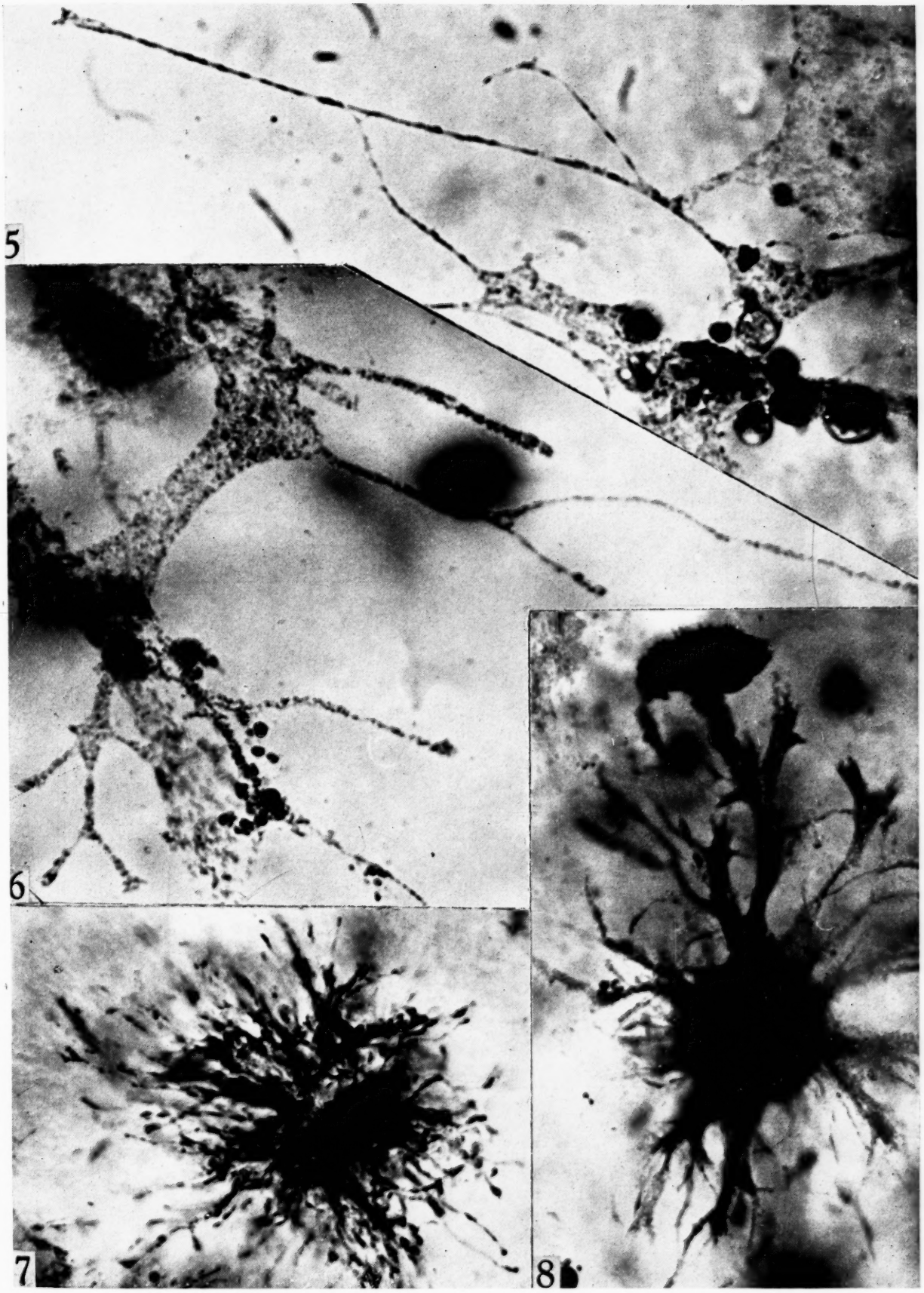

FIG. 5.—A living dendritic melanoblast at the margin of the explant, showing accumulation of melanin granules in the extended processes.

FIG. 6.—A living melanoblast at the margin of the explant. The extended dendrite shows various amounts of melanin. There is some indication of clasmatosis (lower left).

FIGS. 7 and 8.—Two living melanophores within the explant.

round, and appeared as black spheres; this condition remaining until the cell disintegrated.

sumption of ameboid shapes the cells migrate but their movement is limited. Their cytoplasm is filled with

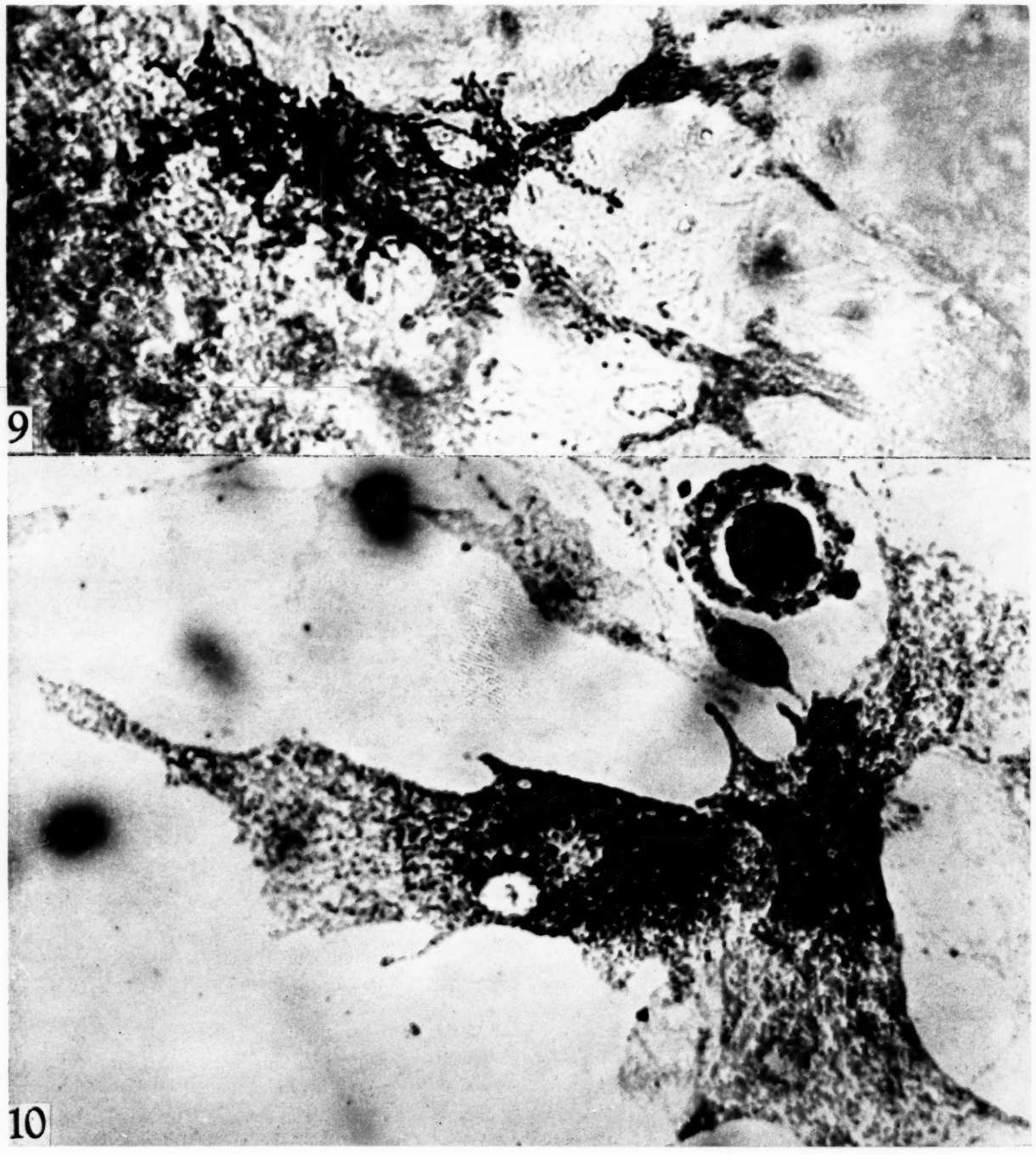

FIG. 9.—Living melanophore at the margin of the explant, showing progressive loss of the extended processes and assumption of ameboid shape.

FIG. 10.—Two living melanophores in the medium away from the explant, showing loss of dendrites and assumption of ameboid shapes.

Several melanophores which appeared in the culture medium away from the explant were characterized by a striking change in the shape of their processes. Fig. 9 shows a melanophore at the margin of the explant where it had assumed an ameboid shape. With assumption of ameboid shapes the cells migrate but their movement is limited. Their cytoplasm is filled with minute granules of pigment which in some cases may accumulate in that part of the cell farthest from the extending pseudopodia. Fig. 10 shows two greatly outspread melanophores. The relatively small round-oval nucleus can be seen in the lower part of one of the cells.

In the early stages of melanosis the melanophores were numerous while in the advanced stage of the neoplasm they were few. With the decrease in the number of melanophores in the later stages there was a corresponding increase in number of the melanoblasts which became the preponderate cell type.

Xanthophores.—In culturing fragments of tissue from fish in the early stages of melanoma development, a number of yellow pigmented cells or xanthophores appeared. In some of these xanthophores the yellow pigment could be definitely seen to be restricted to fine granules dispersed throughout the cell. In others the color seemed to pervade the entire substance of the cell giving it a homogeneously yellow appearance.

Discussion

The neoplastic nature of the melanotic fish growths from material of adult fish is indicated by the fact that the material in tissue culture grows far more readily than does the normal tissue of the same or similar fish. It is well known that this proliferative characteristic is common to neoplasms and to embryonic tissues.

The spontaneously arising melanomas in the spotted platyfish hybrids are evoked by the extreme hypertrophy of macromelanophores in the integument. The significant feature is that this hypertrophy can be induced genetically. In the purebred fish the development and growth of the melanophores keep within bounds of their physiological functions. In the hybrid a new situation arises. The hypertrophic condition of the melanophores develops out of a combination of factors from two dissimilar parents; a factor, or factors, inherited from one parent, profoundly modifies the growth of the melanophores inherited from the other parent. The genetical aspects of this situation are presented by Gordon (3). The important point to be borne in mind is the intimate relation between the hypertrophy of the melanophores and the development of a malignant melanoma of which the characteristic cell is the melanoblast.

The tissue cultures of the genetically induced melanoma present a variety of cell types of which the preponderant type is the melanoblast. Gordon and Smith have already shown from histological preparations that the characteristic, dendritic, melanin-containing cells in the fish melanoma closely resemble those of human melanoma.

The results of our investigation identify the melanoblast as the characteristic cell of both fish and mammalian melanomas with its slender dendritic processes and its peculiar mode of eliminating its elaborated melanin by clasmatosis (5). In the tissue cultures the resemblances are most striking, including the familiar liquefying tendency and the characteristic rapid proliferation of tumor tissue *in vitro* with the consequent excessive acidification of the medium. In this regard the fish melanoma resembles human melanoma more than it does the Harding Passey mouse melanoma in which excessive melanin production is accompanied with an excessive alkalinity to the extent of inhibiting the growth.

A peculiarity of the piscine melanoma is the presence of the macromelanophores which, in tissue culture, are capable of more or less extensive migration with an accompanying drastic change in the shape of their pseudopodial processes. The large size of these melanophores precludes their being confused with the melanoblasts and the melanin-laden macrophages. The macromelanophores also have never been observed to undergo clasmatosis. Whether they are the normal prototypes of the melanoblasts it is not possible to state at the present time. All that can be stated is that the melanoma arises only when macromelanophores are present and accompanies their hypertrophic proliferation.

Except for the occasional presence of the macromelanophores the fish and mammalian melanoma cultures could not be distinguished morphologically if it were not for the quantitative differences in the types of cells present and certain slight peculiarities in some of the fibrocytes; *viz.*, the fan cells of the fish material. The quantitative differences relate principally to the melanoblasts and to the melanin-laden macrophages, the latter being abundant in mammalian and scarce in fish material; while the melanoblasts, of which there are relatively fewer in mammals, far outnumber both macrophages and fibrocytes in the fish material. Some of the cultures of the fish material were actually almost pure cultures of melanoblasts.

Summary

1. Tissue cultures of a fish melanoma were obtained in which, by using a culture medium containing fish serum, all cell types characteristic of mammalian melanoma could be identified,—macrophages, fibrocytes, and melanoblasts.

2. The melanoblast was the last cell type to migrate from the explant. In its morphology and behavior, including its property of clasmatosis, it is identical with the melanoblast of the mouse and human melanoma.

3. Typical melanophores were also observed in the fish melanoma cultures. In the explant they retained the characteristic radial and dendritic shapes of their pseudopodia but those which left the explant became ameboid. Whether the melanophores, particularly the macromelanophores, are the normal prototypes of the melanoblasts it is not possible to state at the present time.

The authors are indebted to Mr. Fred Flathman, an aquarist of Woodhaven, Long Island, N. Y., for the melanotic hybrids of the albino swordtail and spotted platyfish.

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Communications

A Further Note on the Current Literature of Research

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In a note on the current literature of cancer research in a recent number of this journal (CANCER RESEARCH, 1:164. 1941), the writer pointed out that in 1936 the papers relating to induced lung tumors in the mouse were distributed over 14 journals; the number of such journals must now, in 1941, be still greater. The question was raised whether some concentration of the literature of such a subject into a smaller number of journals could be brought about.

Just after the publication of this note, a letter appeared in *Nature* (Miriam Rothschild, Publication of New Species, *Nature*, **147**:676. 1941) which states in a very forcible and concise manner the extent of this defect in the organization of scientific literature as it affects the zoological systematist. The data are shown in a very instructive table which, in an abbreviated form, is reproduced below.

In year 1935	Number of publications other than books	Number of journals used	Number of authors	Journals publishing one paper only devoted to the one group of animals in question	Journals entirely devoted to one group in question	Number of new species described in the year	Number of journals in which these new species are described
Protozoa *	940	263	605	150	3	549	83
Mollusca	1117	376	698	184	7	1437	139
Helminths (including medical literature)	1400 (392 medical)	451 (166 medical)	1191	287	3	776	112
Siphonaptera (Insecta)	19	17	15	15	0	15	5
Aves	1662	388	1043	203	39	230 †	39

* These groups are not comparable as entities, and include fossil species.
† Subspecies as well as species.

"The table above shows an analysis of the literature of a 12-months' period chosen at random, and relating to five groups of animals, also chosen at random."

The table shows that no less than 139 journals are required for the description of 1437 new species of *Mollusca* and 112 journals for the record of 776 new helminths. And further, a student of *Mollusca* who had time, and opportunity, to examine all the 376 journals dealing with this group would find in 184 of them only one paper which concerned him in the course of a whole year's issue. The enormous disparity between the numbers of journals dealing with, and devoted wholly to, any one group (376, and 7, respectively in the case of *Mollusca*) shows the scope that there is for at any rate some degree of segregation of subjects in scientific literature.

A part of this evil must be due to the institutional journals which publish papers concerned with a very wide range of subjects and hence increase the dispersion of the literature of any one subject. Some of these journals have historical traditions and would not welcome alteration. Any complete solution of these difficulties must be international, and would encounter linguistic and political difficulties; however any such action is out of the question at present. There is ample scope for reform within the bounds of a single country, or of a group of countries using the same language.

The present position is best stated in Rothschild's words: ". . . . however diverse the legitimate and non-legitimate reasons for the scattering may be, the fundamental underlying cause is the total lack of any real system associated with the publication of scientific literature."

Abstracts

Reports of Experimental Research

Carcinogenic Compounds

BACHMANN, W. E., M. CARMACK, and S. R. SAFIR. [Univ. of Michigan, Ann Arbor, Mich.] SOME MODIFICATIONS OF THE SYNTHESIS OF 3,4-BENZPYRENE FROM PYRENE. J. Am. Chem. Soc., 63:1682-1685. 1941.

Investigations have demonstrated that certain modifications may be introduced into the conventional synthesis of 3,4-benzpyrene thereby permitting the simplification of two troublesome stages of the reaction.—H. J. C.

BACHMANN, W. E., and M. CARMACK. [Univ. of Michigan, Ann Arbor, Mich.] 4′,5-DIMETHYLENE-3,4-BENZPYRENE. J. Am. Chem. Soc., 63:1685-1688. 1941.

The polycyclic hydrocarbon, 4′,5-dimethylene-3,4-benzpyrene, which combines in one molecule the structural features of the two potent carcinogens, 3,4-benzpyrene and 20-methylcholanthrene, has been synthesized and is now under test.—H. J. C.

CASON, J., and L. F. FIESER. [Harvard Univ., Cambridge, Mass.] 3,7-DIHYDROXY-1,2,5,6-DIBENZANTHRAQUINONE. J. Am. Chem. Soc., 63:1256-1258. 1941.

Demethylation of synthetic 3,7-dimethoxy-1,2,5,6-dibenzanthraquinone gave the free dihydroxy compound which was converted into the diacetate. Both compounds differed from the corresponding derivatives of a dihydroxydibenzanthracene isolated by Levi and Boyland as a product of metabolism of 1,2,5,6-dibenzanthracene in rabbits which, therefore, is not the 3,7-dihydroxy derivative.—H. J. C.

COWDRY, E. V., and F. X. PALETTA. [Barnard Free Skin and Cancer Hosp. and Washington Univ., St. Louis, Mo.] ALTERATIONS IN NUCLEAR VISCOSITY DURING EXPERIMENTAL CARCINOGENESIS DETERMINED BY ULTRACENTRIFUGATION. Am. J. Path., 17:335-358. 1941.

In this study an ultracentrifuge driven by compressed oxygen and developing 120,000 r.p.m. was used. Small fragments of living tissue were excised and placed without delay in the rotor and centrifuged for 30 minutes. They were then studied histologically. The observed degree of displacement of the nucleoli and chromatin in nuclei of cells of different tissues was believed to be inversely proportional to the relative nuclear viscosity in the different tissues.

Normal mouse epidermis and that undergoing transformation to carcinoma by methylcholanthrene applications was studied. The intranuclear viscosity was high in normal cells, lower in hyperplastic epithelium, and much lower in the squamous cell carcinoma finally produced. A lower intranuclear viscosity was also found in embryonic mouse epithelium than in that of newborn animals, and lower in the latter than in adult epidermis. Corresponding results were observed in a study of human tissues (normal epidermis, regenerating epithelium, papillomas, and squamous cell carcinomas). In all tissues when cells had undergone pyknosis the intranuclear viscosity increased so that no displacement resulted from centrifugation.—H. B.

CREECH, H. J., and R. N. JONES. [Harvard Univ., Cambridge, Mass.] THE CONJUGATION OF HORSE SERUM ALBUMIN WITH ISOCYANATES OF CERTAIN POLYNUCLEAR AROMATIC HYDROCARBONS. J. Am. Chem. Soc., 63:1661-1669. 1941.

A number of conjugated proteins containing carcinogenic hydrocarbons as prosthetic groups were prepared for carcinogenic and immunological studies. Marked differences in the extent of conjugation were observed because of the varying degrees of solubility in dioxane exhibited by the isocyanates. Fractionation experiments indicated that the major portion of the conjugated protein contains close to the average number of prosthetic groups. The characteristic fluorescence of the conjugates in ultraviolet light is recorded.—H. J. C.

CREECH, H. J., and R. N. JONES. [Harvard Univ., Cambridge, Mass.] CONJUGATES SYNTHESIZED FROM VARIOUS PROTEINS AND THE ISOCYANATES OF CERTAIN AROMATIC POLYNUCLEAR HYDROCARBONS. J. Am. Chem. Soc., 63:1670-1673. 1941.

Bovine serum albumin was found to undergo conjugation with the isocyanates of carcinogenic hydrocarbons to the same extent as did horse serum albumin whereas only a small number of hydrocarbon prosthetic groups could be introduced into egg albumin, bovine and horse serum pseudoglobulins without causing marked denaturation of the protein component. No significant degree of coupling was observed with zein. A new type of 'labelled' antibody exhibiting an intense blue fluorescence in ultraviolet light was prepared from an antipneumococcus serum.—H. J. C.

GYORGY, P., E. C. POLING, and H. GOLDBLATT. [Babies and Childrens Hosp. and Western Reserve Univ., Sch. of Med., Cleveland, Ohio] NECROSIS, CIRRHOSIS AND CANCER OF LIVER IN RATS FED A DIET CONTAINING DIMETHYLAMINOAZOBENZENE. Proc. Soc. Exper. Biol. & Med., 47: 41-44. 1941.

A control group of rats were fed basal diet A which consisted of rice, carrots, and butter yellow in oil (0.6 gm. per kg. of diet), supplemented with 20 μgm. daily each of thiamine, riboflavin, and pyridoxine and with 100 μgm. daily of pantothenic acid. Cirrhosis, atypical, nodular pro-

liferation of bile ducts and liver carcinoma resulted, fluctuating from 80 to 100% in different groups. However, only 40% of rats fed the same diets with 18% casein added showed similar changes.

Sixty rats were put on basal diet A with the same supplement. In addition subgroup 1 (16 rats) of this experiment got 10 to 20 mgm. choline daily and subgroup 3 (12 rats) received daily 25 to 50 mgm. cystine plus 10 to 20 mgm. choline. Subgroup 4 (20 rats) received no supplement. The rats in subgroups 1, 2, and 4 exhibited moderate to marked cirrhosis, atypical nodular proliferation of bile ducts and liver carcinoma, while subgroup 3 showed but slight cirrhosis and bile duct changes and no carcinoma.

Twenty rats were kept on diet B, composed of casein 18%, cane sugar 68%, melted butter fat 8%, salt mixture 4%, and cod liver oil 2%, supplemented with thiamine, riboflavin, pyridoxine (20 µgm. of each daily), pantothenic acid (100 µgm. daily), and butter yellow (0.6 gm. per kg. of diet). These animals were conspicuously free of cancer or atypical bile duct proliferation.

Eighty rats were fed diet C, composed of casein 60%, lard 23%, cane sugar 15%, cornstarch 50%, salt mixture 4%, cod liver oil 2%, supplemented with butter yellow as above. The animals were subdivided into 4 groups. Subgroup 1 (18 rats) received no further supplement, subgroup 2 (30 rats) received 20 mgm. choline daily, subgroup 3 (18 rats) received 50 mgm. cystine daily and subgroup 4 (14 rats) received 50 mgm. cystine plus 20 mgm. choline. All the animals in subgroups 1 and 2 showed evidence of diffuse and severe hemorrhagic necrosis with or without cirrhosis. All the rats in subgroup 3 exhibited marked cirrhosis, whereas in subgroup 4, only 2 rats showed severe cirrhosis, 3 showed slight cirrhosis or necrosis and the remainder were free even of such changes.

It is concluded that the lipotropic activity of casein and especially the combined oral administration of cystine plus choline afford a definite but not regular protection against pathological changes in the liver produced by a diet containing butter yellow.—M. B.

LAW, L. W. [Roscoe B. Jackson Memorial Lab., Bar Harbor, Me.] BILE ACIDS AND PULMONARY TUMOR INCIDENCE IN A STRAIN MICE. Proc. Soc. Exper. Biol. & Med., 47:37-39. 1941.

Three groups of strain A mice, with equal numbers of males and females in each, were used. Group A (30 mice) was given a total dosage of 10 mgm. cholic acid in olive oil. The first injection was given at the age of 1 month and 3 subsequent injections were given within the following 2 months. Group B (20 mice) was given the same dosage of sodium desoxycholate in the same manner. Group C (38 mice) served as controls. The animals were killed at 9 months of age. Lung nodules were looked for grossly with the dissecting microscope and confirmed histologically.

The control mice and those which received cholic acid exhibited the same proportion of lung tumors (49%). Those mice treated with sodium desoxycholate showed an increased incidence of pulmonary tumors (80%). The mean number of nodules per lung was also increased in this group. The effect of larger doses of desoxycholic acid on lung tumor incidence in this strain is being studied.—M. B.

MURPHY, J. B., and E. STURM. [Rockefeller Inst. for Med. Research, New York, N. Y.] FURTHER INVESTIGATION ON THE TRANSMISSION OF INDUCED TUMORS IN FOWLS. Cancer Research, 1:609-613. 1941.

Tests on 8 strains of chicken tumors induced by carcinogenic chemicals have failed to give any evidence of transmissible agents separable from the tumor cells. The methods used for preparing the material included desiccation, filtration, and high speed sedimentation, all of which methods have proved successful in securing the active agents from fowl tumor strains of spontaneous origin. The cell-free products of the induced tumors were tested by 300 inoculations in 150 young fowls of the same breed as that in which the tumors were induced. A report is also included which shows that x-ray given in sufficient amount to damage the tumor cells, but insufficient to inactivate the filterable agents, destroys the transplantability of the induced tumors. This more extensive study confirms earlier experiments which showed that the induced fowl tumors like the tumors in mammals can be transmitted only by intact living tumor cells.—Authors' abstract.

ROFFO, A. H., and B. LUCHETA. [Inst. med. exper. para el estud. y trat. d. cáncer, Buenos Aires] CANCER Y TABACO. MODIFICACIONES HEMÁTICAS DE LOS CONEJOS CON CARCINOMAS PRODUCIDOS POR ALQUITRAN DE TOBACO. [CANCER AND TOBACCO. BLOOD ALTERATION PRODUCED IN RABBITS BEARING CARCINOMAS INDUCED BY TOBACCO TAR.] Bol. Inst. de med. exper. para el estud. y trat. d. cáncer, 55:747-755. 1941.

Roffo has induced cancer in the ears of rabbits by paintings with tobacco tar. The authors describe the changes that take place in the blood cells paralleling the development of malignancy in the skin. The sequence of events is as follows: I. Irritation of the bone marrow with appearance of immature red cells in the blood stream. II. Further irritation with further increase of the immature red cells. III. Diminution and disappearance of the reticulocytes. This phase is coincident with the malignant transformation of the papillomas and development of cancerous cachexia.—M. D-R.

SELLE, W. A., P. BRINDLEY, and J. W. SPIES. [Univ. of Texas, Galveston, Texas] THE PRODUCTION OF TUMORS BY TRANSPLANTATION OF NORMALLY APPEARING LIVER CELLS FROM ANIMALS PREVIOUSLY INJECTED WITH METHYLCHOLANTHRENE. Cancer Research, 1:618-619. 1941.

Freshly excised liver tissue, apparently normal and free from metastases, from mice bearing tumors induced by injections of methylcholanthrene was injected subcutaneously into normal mice. Tumors arose at the site of injection in a number of animals. Cell-free filtrates of the same liver tissue, injected in the same manner did not induce the formation of tumors.—Authors' summary.

SHIMKIN, M. B., and G. B. MIDER. [Nat. Cancer Inst., Bethesda, Md.] INDUCTION OF TUMORS IN GUINEA PIGS WITH SUBCUTANEOUSLY INJECTED METHYLCHOLANTHRENE. J. Nat. Cancer Inst., 1:707-725. 1941.

Guinea pigs of 2 inbred families were injected subcutaneously with 20 to 40 mgm. of 20-methylcholanthrene dissolved in sesame oil. Of 34 animals surviving over 18 weeks, 29 developed tumors at the site of injection. The tumors appeared at an average of 25 weeks after injection. The great majority of the tumors produced were fibrosarcomas, 9 were liposarcomas, and 5 contained

bone or cartilage. Four tumors metastasized into the lungs and 6 of 7 tumors were transplanted successfully into other guinea pigs of the same strain.—L. L. W.

STEELE, R., F. C. KOCH, and P. E. STEINER. [Univ. of Chicago, Chicago, Ill.] THE EXTRACTION OF A CARCINOGENIC FRACTION FROM HUMAN URINE. PRELIMINARY REPORT. Cancer Research, 1:614-617. 1941.

Urines from normal men and from male cancer patients were exhaustively extracted 3 times; first with benzene, next with butyl alcohol, and finally with benzene after boiling for 15 minutes with HCl added to pH 1.1 to 1.2. The materials recovered from the first two extractions were incorporated in sesame oil and injected subcutaneously into albino mice. None of the animals which received the benzene extracts developed tumors at the site of injection. Of the 17 mice which received 1 injection of 100 mgm. of the butyl alcohol-soluble fraction from the normal urine, 16 survived for 14 months, and of these, 2 died with tumors at 21 and 23 months. Eight mice are still alive at the end of 24 months. Of the 17 mice which received 100 mgm. of the butyl alcohol extract from cancer urine, 1 died with a tumor in the 17th month. One other mouse in this group living at that time later died without a tumor. These 3 tumors were fibrosarcomas which developed at the site of injection. They were different from the usual sarcomas induced with the carcinogenic hydrocarbons in four respects; namely, long induction time, slow rate of growth, early ulceration of the overlying skin, and large amount of collagen. An apparent increase in the number of mammary gland, lung, and lymphatic tumors (all also spontaneous in this stock of mice) in these experiments awaits further data for analysis. The butyl alcohol extracts of normal or cancer urine did not retard or accelerate the tumor induction or modify the type of tumor produced by methyl cholanthrene if injected together.—Authors' abstract.

SUGIURA, K. [Memorial Hosp., New York, N. Y.] EFFECT OF FEEDING WHEAT GERM OIL ON PRODUCTION OF LIVER CANCER BY BUTTER-YELLOW. Proc. Soc. Exper. Biol. & Med., 47:17-19. 1941.

Two samples of wheat germ oil were used; sample I was obtained by continuous Soxhlet extraction with ether for 24 to 48 hours at 50° C. 1000 gm. of wheat germ yielded about 70 cc. of a yellowish brown oily emulsion. Sample II was prepared by the Ward Baking Co., 100 cc. of the oil representing 900 gm. of wheat germ.

Three groups of rats of the Sherman strain were used: Group A. 10 rats were kept on a mixture of 95% unpolished rice and 5% wheat germ oil (sample I); Group B. 10 rats were fed a mixture of 95% unpolished rice and 5% wheat germ oil (sample II); Group C. 20 rats were kept on unpolished rice without wheat germ oil. Each rat received a small amount of fresh carrot daily, and ate about 5 to 7 gm. of food daily. Twenty cc. of a 3% solution of butter yellow in olive oil per 1000 gm. of food was given to all the animals. After 150 days all surviving rats were sacrificed and examined. Gross diagnosis was confirmed by microscopic examination.

The data show that daily ingestion of wheat germ oil had no protective effect upon the production of liver cancer. Of the 16 rats which died or were killed between 100 to 150 days, liver cancer, both cholangioma and hepatoma, were present in 15 (94%).—M. B.

WILSON, R. H., F. DeEDS, and A. J. COX, JR. [Stanford Univ. Sch. of Med., San Francisco, Calif.] THE TOXICITY AND CARCINOGENIC ACTIVITY OF 2-ACETAMINOFLUORENE. Cancer Research, 1:595-608. 1941.

2-Acetaminofluorene was found to have no demonstrable acute toxicity for rats, in quantities up to 50 mgm. per kg. of body weight, subcutaneously, and 1 gm. per kg. gastrically. However, the compound was found to be very toxic when incorporated in the food and fed continuously to rats.

Female albino rats failed to survive 100 days when the concentration of 2-acetaminofluorene in the food was 0.25% or greater, whereas male albino rats died in the same period on concentrations of 0.062% or more. Normal growth rates were not observed if the concentration of 2-acetaminofluorene in the diet exceeded 0.016%. The more severely poisoned rats living for 100 days had livers which were rough, yellowish, and slightly, though definitely heavier than normal.

Feeding albino rats for a period of 95 days or more a diet containing 0.031% or more of 2-acetaminofluorene led to an irregular epithelial hyperplasia of a number of organs, especially the bladder, renal pelvis, liver, pancreas and lung. All animals showed this change to some degree, but not necessarily in all organs. In 19 of the 39 rats fed the compound for this length of time, tumors developed which were classified as malignant by virtue of their invasive growth. The tumors were carcinomas in all but 3 rats. There was 1 sarcoma and in 2 animals the liver lesions resembled leukemic infiltrations. The tumors were multiple in several of the animals and in 3 of these were considered metastatic.—Authors' abstract.

Hormones

DOISY, E. A., JR., M. N. HUFFMAN, S. A. THAYER, and E. A. DOISY. [Lab. of Biol. Chem., St. Louis Univ. Sch. of Med., St. Louis, Mo.] SOLUBILITIES OF SOME ESTROGENS. J. Biol. Chem., 138:283-285. 1941.

The authors record the solubilities at 10 and 30° C. of purified samples of estriol, estrone, estradiol, and equilenin in the following carefully purified solvents, methanol, ethanol (95%), butanol, butyl ether, benzene, toluene, acetone, chloroform, dioxane, ligroin, and pyridine.—H. J. C.

GARDNER, W. U. [Yale Univ. Sch. of Med., New Haven, Conn.] ESTROGENIC EFFECTS OF ADRENAL TUMORS OF OVARIECTOMIZED MICE. Cancer Research, 1:632-637. 1941.

Tumors of the adrenal glands arose in 13 of 15 ovariectomized mice of the early generations of the NH strain. The tumors weighed from 158 to 146 mgm. in 3 mice. The adrenal glands of 6 mice were two or more times the usual dimensions; in some animals they weighed 30 to 50 mgm. The tumors consisted of cords or, in one case, follicular structures of small hyperplastic cells. The smaller tumors showed some indication of cellular differentiation, since large vacuolated cells frequently intermingled with the cords or islands of smaller cells.

These adrenal tumors were associated with evidences of estrogenic stimulation as determined by the condition of the uteri, pelves, and mammary glands.—Author's abstract.

HEIMAN, J. [Coll. of Physicians and Surgeons, Columbia Univ., New York, N. Y.] THE EFFECT OF ANDROGENS AND ESTROGENS ON SPONTANEOUS BENIGN MAMMARY TUMORS IN THE RAT. Am. J. Cancer, 40:343-354. 1940.

Spontaneously occurring adenofibromas and fibromas of the mammary glands of rats were removed and transplanted into the same animals and others from which similar tumors had been removed. Such auto- and homotransplants were carried through several passages and showed no morphological alterations. However, when the animals bearing such transplants were injected with estrogens or androgens or a combination of both, the structure of the tumors was altered as follows: With the injection of estradiol benzoate (dose 0.1 to 2.5 mgm.), the epithelial elements of transplanted adenofibroma were definitely stimulated so that the tumor appeared microscopically as an adenoma or cystadenoma. Androgens, on the other hand, (dose 5 to 15 mgm. testosterone proprionate) inhibited epithelial proliferation in the tumors, resulting in the production of slow-growing fibromas. Further, the androgenic substance moderately inhibited the growth of spontaneous mammary fibromas. When injected together in the ratio of 1:10, estrogens and androgens stimulated the growing epithelium of adenofibromas much as did estrogens alone.—L. L. W.

MARINE, D., and S. H. ROSEN. [Montefiore Hospital, New York, N. Y.] SEX HORMONES AND LYMPHOMATOSIS IN FOWLS. Proc. Soc. Exper. Biol. & Med., 47:61-62. 1941.

Forty-one white leghorn male castrates of the same age were divided into 3 groups: I. 12 birds, each receiving 1 mgm. estradiol dipropionate weekly; II. 12 birds, each receiving testosterone dipropionate in variable amounts twice weekly; III. 17 controls. After 6 months, 4 (33⅓%) of the androgen group; 7 (58%) of the estrogen group; and 4 (23%) of the control group had died of lymphomatosis. Routine injections were still being given to survivors.

Biopsies showed the liver may be transformed from the normal (about 40 gm.) reddish brown color to a huge (300 gm.) reddish gray organ in 15 days.

The polymorphism of lymphomatosis was found in all 3 major groups of leukosis (mycloid, erythro, and lymphoid). Routine blood smears at monthly intervals or oftener were consistently negative for leukemia. The increased occurrence of lymphomatosis in the estrogen-treated group suggests that the induced sex hormone imbalance may have been a factor in stimulating or activating the agent responsible for lymphoblastic tissue overgrowth.—M. B.

MUUS, J., A. H. COONS, and W. T. SALTER. [Thorndike Memorial Lab., Boston City Hosp. and Dept. of Med., Harvard Med. Sch., Boston, Mass.] THYROIDAL ACTIVITY OF IODINATED SERUM ALBUMIN. J. Biol. Chem., 139:135-143. 1941.

When serum albumin was iodinated in stages, thyroidal activity appeared at 6% iodine and increased up to a 10% iodine content. Further iodination did not enhance the metabolic activity of the preparation. The results suggest that diiodotyrosine must be formed before metabolic activity appears and that a completely active iodoprotein requires the introduction of at least one additional iodine atom per molecule of protein.—H. J. C.

SHIMKIN, M. B., and H. B. ANDERVONT. [Nat. Cancer Inst., Bethesda, Md.] EFFECT OF FOSTER NURSING ON THE RESPONSE OF MICE TO ESTROGENS. J. Nat. Cancer Inst., 1:599-605. 1941.

The theory that certain strains of mice have a high incidence of mammary carcinoma because they are relatively resistant to estrogens and must, therefore, elaborate more of the hormone to accomplish estrus, was tested by foster nursing experiments. If the "milk influence" can bring about a changed susceptibility to mammary carcinoma, it might do this by altering the amount of estrogen produced in a given animal—a fact readily determined by the examination of vaginal smears.

While the authors found that the strains of mice used, C3H, C, and C57 were resistant to the action of estrogens in inverse ratios to their mammary cancer susceptibilities, foster nursing among the strains did not change the reactions to the estrogenic hormone. Changes in tumor incidence was marked, however.

In a corollary experiment foster nursing of low tumor strain (C) mice by high tumor strain (C3H) mothers was found to increase the susceptibility of the C mice to mammary tumors induced with estrogens. The authors feel from these experiments that the hormonal factor in the production of mammary tumors in mice is of secondary importance.—L. L. W.

WOGLOM, W. H. [Columbia Univ., New York, N. Y.] CASTRATION AND SARCOGENESIS. Am. J. Cancer, 40: 321-323. 1940.

In an effort to settle the vexed question as to whether or not castration affects the susceptibility of mice to induced tumors, the author castrated 260 male mice (Dobrovolskaïa-Zavadskaïa R III strain) and set aside an equal number of controls of a similar age. The castrated and control mice were then divided into 3 groups which were injected subcutaneously with 0.25 mgm. methylcholanthrene in warm paraffin 2, 4, and 6 months later. Omitting all mice in which ulceration occurred or which died before the first tumor appeared, 192 castrates and 181 intact controls remained. Of the castrates, 139 bore tumors, 72%, and 111 of the controls, 61%. There was considerable discrepancy between the castrates and controls in the group injected 2 months after castration, but the groups injected at 4 and 6 months showed very small differences between castrates and controls. Therefore, the author concludes that prepuberty castration does not change the susceptibility of mice to induced methylcholanthrene sarcoma, and stresses the need of using large number of animals in making such determinations.—L. L. W.

Genetics

GORDON, M. [The Aquarium, New York Zool. Society, New York, N. Y.] GENETICS OF MELANOMAS IN FISHES V. THE REAPPEARANCE OF ANCESTRAL MICROMELANOPHORES IN OFFSPRING OF PARENTS LACKING THESE CELLS. Cancer Research, 1:656-659. 1941.

A black-spotted *Platypoecilus maculatus* was mated to an albino *Xiphophorus hellerii;* they produced melanomatous hybrid offspring. The platyfish parent visibly carried macromelanophores; the swordtail parent had no visible melanophores. The hybrid offspring had not only macromelanophores but micromelanophores as well. Micro-

melanophores appeared in the hybrids owing to the interaction of the platyfish factors, *II stst*, with those of the swordtail, *ii StSt*. The melanomas are produced by the interaction of the platyfish factor, *Sp*, with the *A* and *B* factors of the albino swordtail. The constitution of the melanomatous hybrids is as follows: *Ii Stst Spsp Aa Bb*. These fish were the source material for the tissue culture studies of piscine melanomas.—Author's abstract.

Radiation

APPEL, M., O. SAPHIR, and A. A. STRAUSS. [Michael Reese Hosp., Chicago Ill.] MORPHOLOGICAL ALTERATIONS IN THE REGRESSING BROWN-PEARCE TUMOR AND THEIR RELATION TO CHANGES DUE TO IRRADIATION. Arch. Path. 31:317-325. 1941.

The gross and cytologic characteristics of resorbing Brown-Pearce carcinoma transplants in refractory rabbits, and resorbing tumors in rabbits rendered resistant or immune by intracutaneous transplantation, are described, and certain striking similarities to the changes following irradiation of tumors with divided doses are pointed out. Various theories explaining the mechanism of the production of this immunity are discussed. There is no evidence that anything is liberated by or contained within the regressing tumor that might be considered responsible for the regression, for regressing tumors transplanted into the testes of normal rabbits give the same results as transplants of actively growing tumors.—H. G. W.

FOGG, L. C. and S. WARREN. [Boston Univ. Sch. of Med. and Deaconess Hosp., Boston, Mass.] SOME CYTOLOGIC EFFECTS OF THERAPEUTIC RADIATION. Cancer Research, 1:649-652. 1941.

Cytologic studies were made of the effect of radiation in doses of 2,400 r and 4,800 r on the mouse sarcoma CR 180 and rat carcinoma Walker 256. Attention was centered on a quantitative study of the effect of varied doses of radiation on the frequency of multiple centrioles and the appearance of abnormal mitoses. Counts were made of normal and multiple centrioles at stated periods after radiation (18, 24, 48, 72, 96, and 120 hours).

In mouse sarcoma 180 (dose 2,400 r) there is a progressive increase in the number of interkinetic cells which show multiple centrioles up to 96 hours. After that the percentage decreases. This agrees in general with changes reported in Walker rat carcinoma 256. The higher dose produces fewer multiple centrioles.

Regardless of the doses used approximately 90% of the observed mitoses showed gross aberrations up to 96 hours after radiation. The number of abnormal mitoses reaches its maximum soon after radiation while for centrioles the maximum is not reached until several cell generations have been produced.

It is suggested that as a result of radiation most of the cells suffer injury, lethal in some, transient in others. Among the cells which initiate division before recovery a consistent variation in the number of abnormal mitoses and cells with multiple centrioles appear. This is dependent on dose and time elapsed.—Authors' abstract.

MARSHAK, A., and L. A. ERF. [Univ. of California, Berkeley, Calif.] EFFECTS OF INJECTIONS OF NUCLEI ON "TAKE" OF IMPLANTS OF A LYMPHOMA IN MICE. Proc. Soc. Exper. Biol. & Med., 46:428-430. 1941.

The material used was the Gardner-Lawrence lymphoma carried by transplant in the Strong A albino mouse. Four types of suspensions were injected subcutaneously: I. About 500 viable lymphoma cells; II. fragmented lymphoma cells prepared by successively freezing in liquid air, grinding and thawing 3 times; III. nuclei of lymphoma cells; and IV. nuclei of livers of A strain mice. The material of I and II was suspended in 0.9% saline. The nuclei of III and IV were first suspended in 5% citric acid which was later changed to isotonic McIllvaine buffer (pH 7.0) to prevent local ulceration.

In group I, of 100 mice used, only 2 survived, a number too small to permit any deductions. In group II 4 of 43 survivors (9.3%) showed "immunity" to 3 successive implantations. Group III showed 2.4% "immunity," while in group IV there was 4.8%.

Since no failures to "take" were observed in 2,900 control animals inoculated with the tumor, the incidence of immune animals in this strain must be less than 0.035%. As a result of the treatment, this figure was raised to 2.4 to 9.3%, possibly significant.

Since the immunization secured in III and IV are attributable to nuclear material alone, this suggests that the immunization obtained by injection of various types of whole cells and macerated tissues may be referable to the nuclei they contain.—M. B.

REINHARD, M. C., S. G. WARNER, and H. L. GOLTZ. [N. Y. State Inst. for the Study of Malignant Diseases, Buffalo, N. Y.] FURTHER STUDIES ON THE EFFECT OF X-RAYS ON A TUMOR OF KNOWN GENETIC CONSTITUTION. Cancer Research, 1:653-655. 1941.

In an endeavor to produce a genetic change in a transplantable mouse tumor by means of radiation, Simpson tumors were exposed *in vivo* to doses of radiation varying from 25 to 1,000 r. One week following radiation these tumors were transplanted into a strain of mice (C57 black) which fails to grow the nonradiated tumor. The results of this experiment are presented in a graph with the percentage takes plotted against dose, the percentage probable error being shown for each point on the graph. The percentage takes increases from 34% for a dose of 25 r to approximately 70% for a dose of 400 r, while for doses above 400 r there is a decrease in the percentage takes. Following doses of 200 r tumors were transplanted into blacks at varying time intervals. The percentage takes remained constant for a period of 0 to 7 days.—Authors' summary.

Biochemistry and Nutrition—Chemotherapy

COHEN, P. P., and G. L. HEKHUIS. [Yale Univ. Sch. of Med., New Haven, Conn.] TRANSAMINATION IN TUMORS, FETAL TISSUES, AND REGENERATING LIVER. Cancer Research, 1:620-626. 1941.

A series of 6 different mouse tumors, regenerating rat liver, and fetal kitten, and adult cat tissues were investigated for their transaminase activity. The following reactions were studied:

1) glutamic acid + oxaloacetic acid $\underset{b}{\overset{a}{\rightleftharpoons}}$ α-ketoglutaric acid + amino acid.

2) l(+)-glutamic acid + α-keto acid $\underset{b}{\overset{a}{\rightleftharpoons}}$ α-ketoglutaric acid + aspartic acid.

With the tumors, reaction 2a did not proceed at a measurable rate with pyruvic acid as the α-keto acid.

In the case of reaction 2b, the amino acids l(+)-alanine, l(−)-phenylalanine, dl-methionine, l(+)-arginine and l(+)-tryptophane were not active. Some activity was observed with l(−)-aspartic acid (reaction 1b).

The transaminase activity as measured by reaction 1a was found to be low for all the mouse tumors and fetal tissues. Kitten (6 days old) and regenerating liver tissue showed lower values than adult tissues. d(−)-Glutamic acid was not active in reaction 1a with 3 mouse tumors.

The possible significance of a low transaminase activity in relation to rapid growth (protein synthesis) is discussed.—Authors' abstract.

GREENSTEIN, J. P., W. V. JENRETTE, G. B. MIDER, and J. WHITE. [Nat. Cancer Inst., Bethesda, Md.] CHEMICAL STUDIES ON THE COMPONENTS OF NORMAL AND NEOPLASTIC TISSUES. V. THE RELATIVE ARGINASE ACTIVITY OF CERTAIN TUMORS AND NORMAL CONTROL TISSUES. J. Nat. Cancer Inst., 1:687-706. 1941.

Methods are described for determining and expressing the relative arginase activity of various tissues. Comparisons of this activity were made among three kinds of tumors and their control normal tissues. The tumors used included transplanted hepatomas and carcinomas of rats and mice, spontaneous mammary carcinomas of mice, and mouse lymphomas. It was found that the arginase activity of transplanted hepatic tumors (and embryo livers) is much less than that of normal livers. The transplant had no effect on the arginase activity of the liver of the same animal. Regenerating and normal adult livers showed approximately the same activity. Spontaneous mammary tumors were much more active in respect to arginase than were control tissues but lymphomas were only slightly so. Lactating and hyperplastic mammary tissues had about the same arginase activity. While added manganese did not affect the relative activity of hepatic tumor and liver, prolonged dialysis of liver extracts led to a preparation which was strongly activated by the metal. Dialysis of hepatic tumor, however, resulted in almost complete loss of enzyme activity.—L. L. W.

HAMMETT, F. S. [The Lankenau Hosp. Research Inst., Philadelphia, Penn.] THE INFLUENCE OF l-PROLINE ON THE GROWTH OF SPONTANEOUS TUMORS IN THE MOUSE. Growth, 5:69-83. 1941.

Subcutaneous intrascapular injections of the amino acid l-proline in nontoxic concentrations gave an increase in cellular and architectural differentiation of the tumors, an increase in secondary tumor production, a decrease in maximum tumor volume, and some prolongation of life.—J. J. B.

HAMMETT, F. S. [The Lankenau Hosp. Research Inst., Philadelphia, Penn.] THE INFLUENCE OF dl-METHIONINE SULFOXIDE ON THE GROWTH OF SPONTANEOUS TUMORS IN THE MOUSE. Growth, 5:85-111. 1941.

The injection of dl-methionine sulfoxide did not retard the growth of spontaneous tumors in mice.—J. J. B.

KABAT, E. A., and J. FURTH. [Cornell Univ. Med. Coll., New York, N. Y.] A HISTOCHEMICAL STUDY OF THE DISTRIBUTION OF ALKALINE PHOSPHATASE IN VARIOUS NORMAL AND NEOPLASTIC TISSUES. Am. J. Path., 17:303-318. 1941.

This is a study of the presence of alkaline phosphatase in normal and malignant tissues as determined by the histochemical methods of Takamatsu (1939) and Gomori (1939). Large amounts of phosphatase were found in osteogenic tissue, renal epithelium, and small intestine epithelium; smaller amounts were present in endothelium of vessels, certain nerve cells and fibers, and other tissues.

Large amounts were present in the osteoblasts of a transmissible chicken sarcoma and in a malignant mouse sarcoma, but not in nonbone-forming tumors of these animals. The tumor cells in a butter-yellow liver carcinoma of a rat contained some of the enzyme. The epithelial cells of a human fibroadenoma of the breast contained large amounts, but mammary carcinoma cells did not. A Wilm's tumor contained a moderate amount while the other human tumors used possessed none.—H. B.

MAVER, M. E., J. M. JOHNSON, and J. W. THOMPSON. [Nat. Cancer Inst., Bethesda, Md.] THE PEPTIDASE ACTIVITIES OF THE CATHEPSINS OF NORMAL RAT TISSUE AND OF JENSEN RAT SARCOMA. J. Nat. Cancer Inst., 1:675-686. 1941.

In an effort to see if catheptic preparations from tumor tissues and from normal tissues of tumor-bearing rats differed in their peptidase activities from the cathepsins of normal tissues of normal animals, various digestion combinations were set up. The Jensen sarcoma cathepsin showed no specific activity against the synthetic peptides used. All of the catheptic preparations, when activated by cysteine, were able to hydrolyze oxidized glutathione. Under anaerobic conditions they likewise hydrolyzed glutathione extensively. No hydrolysis of peptides containing optically unnatural forms of amino acids occurred with any of the tumor or normal tissue cathepsins.—L. L. W.

Immunology

EISEN, M. J., and W. H. WOGLOM. [Dept. of Cancer Research, Columbia Univ., New York, N. Y.] THE NONSPECIFIC NATURE OF INDUCED RESISTANCE TO TUMORS. Cancer Research, 1:629-631. 1941.

The nonspecific nature of induced resistance to propagable tumors is demonstrated by a series of experiments employing a transplantable mammary adenocarcinoma in 2 inbred strains of rats. Transplants of the tumor grow progressively in 100% of the animals of the August strain in which it had originated spontaneously. This strain was derived from a cross between animals of 2 inbred strains (lines 990 and 1561). Of 41 control rats of line 990, 32 were successfully inoculated with the tumor, and 9 (21.9%) were naturally resistant. The injection 13 days prior to transplantation of 0.3 cc. of embryonic skin derived from August animals into rats of this line failed to induce resistance to grafts. No significant degree of refractivity to the tumor was elicited by embryonic skin of line 990 in rats of the same line. In this group 8 (36.2%) of 22 treated rats were resistant. On the other hand, embryonic tissue of August rats was highly effective in animals of line 990, as 37 (92.5%) of 40 animals of this group failed to grow the tumor. Embryonic tissue, therefore, genetically identical with the tumor, possessed the capacity to immunize against it only in rats partially alien as regards both the embryonic skin and the corresponding tumor. Acquired resistance to transplantable tumors depends upon genetic differences between animals inoculated and the one that

produced the neoplasm. It is not directed specifically against the malignant cell.—Authors' abstract.

FRIEDEWALD, W. F., and J. G. KIDD. [Rockefeller Inst. for Med. Research, New York, N. Y.] DISTINCT TYPES OF ANTIBODIES IN THE BLOOD OF RABBITS CARRYING THE TRANSPLANTED V2 CARCINOMA. Proc. Soc. Exper. Biol. & Med., 47:130-132. 1941.

Rabbits carrying the transplanted V2 carcinoma (virus-induced) develop in their blood an antibody capable of neutralizing *in vitro* the Shope papilloma virus and of fixing complement in mixture with it. Further studies have shown that the virus neutralizing and complement-fixing titers of serums from V2 carcinoma rabbits invariably parallel one another. This antibody is directed against the papilloma virus *per se* and is identical with the antiviral antibody present in the blood of rabbits with virus-induced papillomas which in turn is identical in rabbits injected with purified papilloma virus.

Two other types of antibodies have also been found in blood of rabbits with V2 carcinoma. Serums from rabbits carrying this tumor not only react with normal tissue extracts but also and to a much greater extent with extracts of the V2 carcinoma. When heated at 65° C. for 30 minutes these serums retain undiminished their capacity to react with the V2 carcinoma antigen, though their ability to react with the normal tissue and heterologous tumor antigens is abolished or markedly diminished.

The V2 antibody does not react with purified suspensions of the papilloma virus, while the antiviral antibody does not react with V2 carcinoma extracts. It is differentiated from the natural antibody found in rabbits carrying V2 carcinoma by its specific affinity for V2 carcinoma antigen and its resistance to heat.

Similar studies have been undertaken with other transplanted cancers.—M. B.

GROSS, L. [Inst. Pasteur, Paris, France] DE LA RELATION ENTRE LA REGRESSION SPONTANÉE D'UNE TUMEUR ET L'ETABLISSEMENT DE L'IMMUNITÉ ANTI-NEOPLASIQUE CHEZ LE LAPIN. [CONCERNING THE RELATION BETWEEN THE SPONTANEOUS REGRESSION OF A TUMOR AND THE ESTABLISHMENT OF THE ANTI-NEOPLASTIC IMMUNITY IN THE RABBIT.] Bull. internat. Acadm. polon. d. sc. et d. lett. Cl. med., 101-106. 1939.

The Brown-Pearce epithelioma was implanted intradermally in 29 rabbits. The resultant tumors were excised in from 6 to 24 days after inoculation. Thereafter the rabbits were reinoculated with the same tumor. All animals in which the cutaneous tumors were excised 10 and more days after their implantation were found to be completely resistant to the reinoculation of this epithelioma. Thus, the spontaneous and complete disappearance of the rabbit epithelioma produced by intradermal inoculation of this tumor seems to be only a secondary sign of the establishment of an anti-epitheliomatous resistance in the host. This establishment of anti-epitheliomatous resistance in the rabbit follows the intradermal inoculation of the tumor, but apparently precedes the spontaneous disappearance of the produced neoplasm.—Author's abstract.

JACOBS, J. L., and J. D. HOUGHTON. [Tufts Coll. Med. Sch. and Boston City Hosp., Boston, Mass.] COMPLEMENT-FIXATION TESTS ON RABBITS WITH BROWN-PEARCE CARCINOMA. Proc. Soc. Exper. Biol. & Med., 47:88-90. 1941.

The rabbits were inoculated by intratesticular injection. Antigens were prepared and the complement fixation tests carried out as described by Kidd (Proc. Soc. Exper. Biol. & Med., **38**:292. 1938).

Of 14 rabbits inoculated with tumor, the serums of 3 out of 6 rabbits with rapidly growing tumors and metastases showed definite complement fixation, as compared with 10 negative normal serums as controls. Two other serums were doubtful and 1 was negative. Five animals with small tumors were all negative; and 3 animals in which tumors grew and then regressed were negative.

Variations were met with in attempts to repeat the experiment. Some normal serums fixed complement, and the tumor extracts used as antigens varied markedly in their complement-fixing properties. Prolonged centrifugation, passage through a Mandl filter, or passage of time reduced the tendency to fix complement. Diluting the antigen to the point where reaction with normal serums was eliminated produced the same result when serums of tumor-bearing rabbits were used. The experiment was repeated with frequent bleeding of the animals from the time of inoculation until death from tumor, all samples of any one animal being tested at the same time.

Such tests were carried out on 12 rabbits. Of 8 animals with growing tumors 3 were doubtful and 4 negative, of 4 rabbits with small tumors all were negative. In no case did a serum negative before tumor inoculation become definitely positive after inoculation when compared against the same antigen in the same test.

The experimental findings are discussed in relation to other findings in the same field. It is significant that the positive or doubtful results were mainly in animals with rapidly growing tumors; but it is in animals with regressing tumors that one might expect to find antibody formation, if such formation conferred any immunity to the tumor. Unless the reactions are haphazard, a number of antigens may be involved. This suggests the possibility that the reactions observed might be due to production of antibodies to altered antigens of rabbit organs, resulting from pathological processes induced by the tumor, rather than to a single tumor or virus antigen.

Should specific tumor antibodies be conclusively demonstrated it would still be essential to exclude the likelihood that the antigen substance is part of the cellular elements of the tumor before any virus analogy could be pressed.—M. B.

Leukemia

CURTIS, M. R., and W. F. DUNNING. [Columbia Univ., New York, N. Y.] TRANSPLANTABLE LYMPHOSARCOMATA OF THE MESENTERIC LYMPH NODES OF RATS. Am. J. Cancer, 40:299-309. 1940.

This is a report of a study of 369 tumors of the cecal region in rats of a colony including several inbred strains and hybrids. The majority of these tumors were lymphosarcomas, involving the mesenteric nodes in the ileocecal region. Ulceration of the cecal mucosa was present in some cases but this was thought to be secondary to the lesions in the lymph nodes and not the reverse. Two strains of lymphosarcoma were found to be transplantable into the subcutaneous tissues of rats related to the hosts of the primary tumor.—L. L. W.

STURM, E. [Rockefeller Institute for Med. Research, New York, N. Y.] INDUCED RESISTANCE TO A TRANSPLANTABLE LYMPHATIC LEUKEMIA IN RATS. Cancer Research, 1:627-628. 1941.

A high degree of resistance against transplanted lymphatic leukemia in rats was induced by a prior injection of homologous normal defibrinated blood or embryonic tissue. The embryonic cells from the Wistar strain were just as effective in the production of resistance as those from another strain, the hooded rats. The results were equally as good when the immunizing injections were given subcutaneously as when given intravenously. As the leukemia inoculations were made intraperitoneally, this result would indicate that the resistance depended on a general reaction rather than a local one.—Author's summary.

Tissue Culture

GRACE, E. J. [Grace Clinic, Brooklyn, N. Y.] THE BEHAVIOR OF TUMORS IN TISSUE CULTURE AT TWENTY-FOUR HOURS. New York State J. Med., 41:459-462. 1941.

The author studied explants of 37 human tumors. No proliferation of benign forms occurred, and the extent of activity exhibited by malignant types roughly paralleled the histologic and biologic criteria of the degree of malignancy of the tumors. The possibility of utilizing tissue cultures as a diagnostic aid is suggested.—M. J. E.

GRAND, C. G., M. GORDON, and G. CAMERON. [New York Univ. and New York Aquarium, New York, N. Y.] NEOPLASM STUDIES VIII. CELL TYPES IN TISSUE CULTURE OF FISH MELANOTIC TUMORS COMPARED WITH MAMMALIAN MELANOMA. Cancer Research, 1:660-666. 1941.

Tissue cultures of a fish melanoma were obtained in which, by using a culture medium containing fish serum, all cell types characteristic of mammalian melanoma could be identified; *viz.,* fibrocytes, macrophages, and melanoblasts. The melanoblasts were specially noted as being similar to those of mouse and human melanoma in their morphology and behavior and in possessing the peculiar property of clasmatosis. Typical fish melanophores were also observed.—Authors' abstract.

ROZYNEK, M. [Abt. für exp. Zellforsch., Path. Inst. Univ. Berlin, Germany] UNTERSUCHUNGEN ZUR DIFFERENZIERUNGSFÄHIGKEIT DES JENSEN-SARKOMS. [STUDIES ON DIFFERENTIATION OF THE JENSEN SARCOMA.] Arch. f. exper. Zellforsch., 24:221-232. 1940.

Addition of heparin to explants of the Jensen sarcoma reduces their growth rate and permits greater cellular differentiation as evidenced by an increased production of intercellular fibrils in cultures of 2 to 3 weeks of age. That this is not a permanent alteration is demonstrated by the reversion of the tumor to its original cellular nature with minimal fibril production when differentiated explants are retransplanted into rats.—M. J. E.

Clinical and Pathological Reports

Radiation—Diagnosis and Therapy

ABESHOUSE, B. S. [Sinai Hosp., Baltimore, Md.] THE DIFFERENTIAL DIAGNOSIS OF RENAL NEOPLASMS AND HYDRONEPHROSIS OR PYONEPHROSIS FROM A PYELOGRAPHIC STANDPOINT. Am. J. Roentgenol., 45:214-220. 1941.

Hydronephrosis associated with renal neoplasms is a relatively uncommon condition, and its presence may make the pyelographic diagnosis of renal tumor difficult if not impossible. Pelvic tumors may cause hydronephrosis by acting as a ball valve obstruction. Parenchymatous tumors may cause obstruction by a single factor or a combination of several factors: compression of the pelvis by the tumor, displacement of the kidney causing constriction of the pelvic outlet, occlusion of the outlet by tumor debris, retraction and fibrosis of peripelvic tissues, and secondary infection of the pelvic mucosa.

The distinctive pyelographic changes associated with renal tumors are pressure deformities involving one or more calices, displacement of the pelvis, and irregular or ill defined outlines of the pelvis and calices. The characteristic changes associated with hydronephrosis are true dilatation of the pelvis and calices with uniformly regular and sharp outlines, and the presence of an intrinsic or extrinsic obstruction in the ureter or pelvis.—E. A. L.

CANTRIL, S. T., F. BUSCHKE, and H. M. PARKER. [Swedish Hosp., Seattle, Wash.] IRRADIATION IN CANCER OF THE CERVIX UTERI. Radiology, 36:534-542. 1941.

Whenever conditions permit, the patient is treated first with radium. The usual procedure is to use 40 mgm. of radium (1 mm. platinum filtration) in an intra-uterine tandem and to give 4,000 mg.-hr. in a single application of 4 to 5 days. Twenty-four hours later the vaginal application is made, the dose also being 4,000 mg.-hr. An 800 kv. machine is used for the roentgen irradiation with a 100 cm. target-skin distance. Two anterior suprapubic and 2 posterior lumbo-sacral ports are commonly used, and, occasionally, 2 ischiosciatic. They are not larger than 10 x 14 cm. and at times are 10 x 10 cm. Between 400 and 500 r, measured on the skin, are given to one port a day. The total dose to each port, measured on the skin, has usually not exceeded 3,500 r.—E. A. L.

CAULK, R. M. [Washington, D. C.] A CONCEPTION OF CANCER AS RELATED TO PREOPERATIVE IRRADIATION. West Virginia M. J., 36:511-516. 1940.

The paper contains general remarks on the value of preoperative radiotherapy in cases of cancer of the uterine fundus, ovary, testicle, bladder, colon, and breast. Radiation is administered during a period of 10 to 14 days, following which it is necessary to wait for approximately 5 weeks until the skin reactions have subsided. Radiotherapy acts to reduce the size of the tumor mass by destroying neoplastic cells and diminishing the intensity of the inflammatory reactions commonly associated with tumors.—M. J. E.

DONALDSON, S. W., and I. SILVERMAN. [St. Joseph's Mercy Hosp., Ann Arbor, Mich.] RADIATION CASTRATION AS AN AUXILIARY TREATMENT TO MAMMARY CANCER: REPORT OF A CASE OF RECASTRATION. Ohio State M. J., 37:238-241. 1941.

Roentgen therapy was administered locally to a woman of 49 years with an inoperable carcinoma of the breast and to the ovaries to abolish their activity as the menstrual

cycles were still normal. The primary tumor disappeared completely and evidence of healing was manifest in skeletal lesions interpreted roentgenographically as of metastatic nature. One year later the opposite breast was involved by an extensive cancer and new bone lesions appeared. On the assumption that recovery had taken place in the ovary or minimal activity had been retained a second series of irradiation was given to the ovaries, but the patient died of pulmonary metastases a short time later.—M. J. E.

GOIN, L. S., J. W. CROSSAN, and J. JELLEN. [Queen of Angels Hosp., Los Angeles, Calif.] LOW-VOLTAGE CONTACT ROENTGEN THERAPY (CHAOUL THERAPY). Radiology, 36:583-587. 1941.

The mechanics, operation, and physical characteristics of radiation of the Philips Metalix Contact Therapy Unit are described, and its clinical use is illustrated.—E. A. L.

HANCOCK, J. D., and J. LOVE. [St. Joseph Infirmary, Louisville, Ky.] DIRECT X-IRRADIATION OF TUMORS. South. Surgeon, 10:39-41. 1941.

Irradiation of exposed tumors offers the advantages of permitting the administration of large doses and obviating exposure of considerable areas of normal tissue and extensive damage to the skin. Three case reports illustrate the technic, but sufficient time has not elapsed to give an evaluation of the ultimate results. Doses of 3,200 to 3,600 r (120 kv., filter 1 mm. Al, distance 10 in.) were administered in a single exposure immediately following the operative procedure. A sterile cannula was employed to direct the beam. In the first patient irradiation was applied through a suprapubic cystotomy wound to the fulgurated base of an excised papilloma, in the second to the femoral and inguinal areas from which were extirpated metastases of a penile cancer, and in the third to the deep tissues of the neck following excision of a metastasis of a cancer of the mouth.—M. J. E.

HURDON, E. [Marie Curie Hosp., London] RADIUM TREATMENT OF CANCER OF THE UTERUS. Am. J. Roentgenol., 45:250-258. 1941.

In treatment of the cervix two 25 mgm. tubes of radium are placed in the canal and three 20 mgm. boxes in the vagina, one in each lateral fornix and one centrally located over the cervix. The total dose is 6,000 to 8,000 r and is given in 3 treatments, each of 24 hours' duration, spaced at intervals of one or two weeks. With this method the dose in the lateral pelvic regions varies from 2,300 to 6,600 r depending partly on how high in the fornices the lateral boxes can be packed. There has been a 35.8% 5-year survival rate.

The central vaginal plaque is omitted in carcinoma of the corpus uteri, and an 8 mgm. tube is placed in each cornu. The duration of treatment is the same as in cervical cancer. The dosage in the critical regions in the corpus varies from 2,640 r on the peritoneal surface of the fundus to 6,600 r in the musculature of the fundus and corpus. The 5-year survival rates have been 62.5% in the technically operable cases and 14.8% in the technically inoperable.—E. A. L.

KAPLAN, I. I. [Bellevue Hosp., New York, N. Y.] IRRADIATION OF BRAIN TUMORS AT BELLEVUE HOSPITAL. 1924-1939. Radiology, 36:588-595. 1941.

This discussion is based on 160 brain tumors, 45 of which were of the pituitary. The medulloblastomas were the most radiosensitive of the gliomas. The spongioblastomas were somewhat sensitive, the astrocytomas and ependymomas slightly so. The oligodendrogliomas were radioresistant. Of the pituitary tumors the basophil adenoma was the most radioresponsive. Surgery is the method of choice for the chromophobe adenomas. The acidophile adenomas are moderately responsive.—E. A. L.

KAPLAN, I. I. [Bellevue Hosp., New York, N. Y.] RADIUM BEAM THERAPY. Am. J. Roentgenol., 45:683-691. 1941.

The mechanical construction and clinical application of a 5 gm. radium pack is described. Brief case reports are presented to illustrate its use.—E. A. L.

LILJENCRANTZ, E., and R. R. NEWELL. [Stanford Univ. Sch. of Med., San Francisco, Calif.] TECHNIC OF IRRADIATION OF CANCER OF THE UTERINE CERVIX, COMBINING RADIUM AND SUPERVOLTAGE ROENTGEN RAYS. Radiology, 36:543-545. 1941.

Roentgen ray therapy is used first in the treatment of these patients, in the belief that the best that can be hoped from x-ray is reduction in the size of the tumor. A beam of a h.v.l. of 2.7 mm. So. is used. The pelvis is treated through 3 ports, at 120° to each other, one suprapubic and two postero-lateral. One port is treated daily. The posterior-lateral ports receive 400 r, measured in air, daily and the suprapubic port 300 r. The total dose measured on the skin is 4,800 r to the postero-lateral ports and 3,600 r to the suprapubic.

Within a few days after the roentgen therapy has been completed a total dose of 3000 mg.-hr. of radium (2 mm. platinum filtration) is given to the cervical canal and lateral vaginal fornices.—E. A. L.

MAYNEORD, W. V., and J. HONEYBURNE. [Roy. Cancer Hosp. (Free), London] A PHYSICAL STUDY OF INTRACAVITORY RADIUM THERAPY. Am. J. Roentgenol., 45:235-249. 1941.

This paper describes investigations into the distribution and intensity of radiation in the vicinity of the radium source as it is used in the treatment of carcinoma of the cervix and body of the uterus. By using the protractor, the integral table or the dose contour method as described and analyzed the dosage rate of radiation in and around the uterus and cervix can be found and isodose surfaces constructed.—E. A. L.

MEIGS, J. V. [Harvard Med. Sch., Boston, Mass.] RADIATION THERAPY IN GYNECOLOGY. New England J. Med., 224:67-72. 1941.

This is a review of radiation therapy of benign and malignant tumors of the female genital tract. The author feels that radium and x-ray are destructive agents and "stimulating doses" are a fallacy. The limitations of radiation therapy are recorded. The superior role of surgery is upheld in vulvar carcinoma, pelvic node metastases of cervical cancer, as well as endometrial, fallopian tube, and ovarian carcinoma. A bibliography of 18 papers is given.—A. M.

MEIGS, J. V. [Mass. Gen. Hosp., Boston, Mass.] THE ROENTGEN TREATMENT OF PATIENTS WITH ADVANCED MALIGNANCY. New York State J. Med., 41:260-269. 1941.

The radiotherapist is frequently called upon to administer radiation to patients with advanced malignant disease for the following reasons: the condition is known to be inoperable but some palliation is expected, for

purely psychic reasons, or for the relief of pain or to check ascites. The results in 100 cases are recorded. The most common lesions in this series were cancers of the ovary, large bowel, uterine cervix, pancreas, and stomach. Histologically the greater number were adenocarcinomas. The tumors were either primarily inoperable or had recurred at least 1 year following an apparently satisfactory resection. Roentgen therapy was administered most commonly with an apparatus of 200 kv., but in some instances radiations at 400 kv. or 1000 kv. were employed. An attempt was made to give an adequate fractionated dosage (total of 3,600 to 4,000 r through several portals), as the result appeared superior to those obtained with smaller amounts of radiation. Radiation sickness occurred in 40% of the patients and produced annoying discomfort. The results frequently proved disappointing—33% of the patients did not survive more than 2 months. Not uncommonly the general condition deteriorated seriously. Eleven of the 100 patients were alive at the time of publication of the report, 6 to 9 years following the institution of radiotherapy.—M. J. E.

MOONEY, B. R. [Winnipeg, Canada] CARCINOMA OF THE BREAST: SOME OBSERVATIONS ON PREOPERATIVE X-RADIATION. Canad. M. A. J., 43:580-584. 1940.

This is a discussion of the technic of preoperative roentgen therapy of breast cancer.—M. J. E.

NEWCOMER, E. [Denver, Col.] COMMENTS ON THE TREATMENT AND SEQUELAE OF CARCINOMA OF THE UTERUS. Am. J. Roentgenol., 45:651-660. 1941.

The classification of carcinoma of the cervix and corpus uteri, methods of treatment with x-ray and radium, and complications from these diseases are discussed.—E. A. L.

PFAHLER, G. E. [Grad. Sch. of Med., Univ. of Pennsylvania, Philadelphia, Penn.] TREATMENT OF CANCER OF THE LIP AND MOUTH. Radiology, 35:598-605. 1940.

The greatest opportunity in this field lies in the education of the public and in the alertness of the physician in the recognition and proper therapy of the precancerous lesion, such as erosions, ulcerations, leukoplakia, and papillomas. Electro-desiccation or local applications of silver nitrate have been effective in such lesions.

Carcinomas of the lip less than 0.5 cm. in diameter are treated with electro-desiccation and removal with scissors. The larger tumors are treated either with electrosurgery, radium moulage, or implantation of radium needles. The mental, submental, and submaxillary regions are given prophylactic irradiation. Cancer of the mouth is best treated with irradiation. Lymph nodes with metastatic tumor from carcinoma of the mouth are treated with external irradiation, using either radium packs or high voltage x-rays, and interstitial radium needles. There has been a 98% 5-year cure rate in carcinomas of the lip less than 1.5 cm. in diameter; a 68% 5-year rate in the larger ones; and a 48% rate in the ones with palpable nodes.

Twenty-nine % of the carcinomas of the mouth have survived 5 years. In both of these groups the patients who have not been traced or who have died of intercurrent disease are excluded in arriving at the statistics.—E. A. L.

POWELL, E. V. [Forth Worth, Tex.] PREOPERATIVE RADIATION OF BREAST CANCER. Texas State J. Med., 36:620-622. 1941.

Case records are given of 5 patients with cancer of the breast treated by preoperative roentgen radiation and mastectomy who were tumor-free 4 to 12 years after the conclusion of therapy.—M. J. E.

ROFFO, A. E., and G. IACAPRARO. [Inst. de med. exper. para el estud. y trat. d. cáncer, Buenos Aires] ENSAYOS TERAPÉUTICOS Y RESULTADOS INMEDIATOS CON ROENTGENTERAPIA ULTRAPROFUNDA 400,000 VOLTS—EN LOS CARCINOMAS DE LA PRÓSTATA. [RESULTS WITH ULTRADEEP ROENTGENTHERAPY, 400,000 VOLTS IN CARCINOMAS OF THE PROSTATE.] Bol. Inst. de med. exper. para el estud. y trat. d. cáncer, 55:857-867. 1941.

Report of 4 treated cases in which, despite the fact that the process was much advanced, encouraging results were obtained. Patients not previously operated upon and bearing lesions not yet infected respond best to treatment.—M. D-R.

ROFFO, A. E. JR. [Inst. de med. exper. para el estud. y trat. cáncer, Buenos Aires] LA INSTALACIÓN DE TELEROENTGENTERAPIA DE 600,000 VOLTIOS. [THE USE OF TELEROENTGENTHERAPY OF 600,000 VOLTS.] Bol. Inst. de med. exper. para el estud. y trat. de cáncer, 55:779-794. 1941.

The characteristics of the radiation are: minimum wave 0.0257 A.; effective length 0.068 A.; half value layer of copper 1.65 cm. An additional filter of 1 mm. lead, 5 mm. iron and 3 mm. aluminum is used for the applications. The focal skin distance is 1.5 meters. The deep dose, measured in the paraffin phantom, is 10 cm. depth and 68% of the total incidental radiation. The patients selected for treatment were cases of lung, mediastinum, esophagus, and rectum carcinoma, all with generalized metastases. The treatment induced no untoward symptoms, the only manifestation being a lowering of the pH of the blood.—M. D-R.

ROFFO, A. E. JR. [Inst. de med. exper. para el estud. y trat. d. cáncer, Buenos Aires] ALGUNOS RESULTADOS DE LA ROENTGENTERAPIA ULTRAPROFUNDA CON RADIACIONES PRODUCIDAS CON UNA DIFERENCIA DE TENSIÓN DE 400,000 VOLTS. [SOME RESULTS OF THE ULTRADEEP TELEROENTGENTHERAPY WITH A RADIATION PRODUCED AT 400,000 VOLTS.] Bol. Inst. de med. exper. para el estud. y trat. d. cáncer, 55:809-856. 1941.

This treatment has lengthened by at least several months or years the life of patients who without it would have been considered hopeless cases. Some of them could even be considered as cured because for more than 2 years after the end of the treatment they showed no symptoms of disease. Among these cases are included 1 case of carcinoma of the pancreas, 2 of adenocarcinoma of the breast, 3 of the bladder, 2 of the prostate, 1 of the uterus, and 1 of the esophagus. In 18 other single cases the clinical condition also seems to suggest a permanent cure but the time elapsed since the end of the treatment is only 1 year or less. All the patients were very advanced cases as shown by the 16 appended photographs in which the lesions are shown before and after treatment.—M. D-R.

RUBENFELD, S., and L. D. SCOTT. [Bellevue Hosp., New York, N. Y.] X-RAY TREATMENT OF LEUKEMIAS. Radiology, 36:352-355. 1941.

This is an analysis of 117 cases of leukemia treated from 1925 through 1938. Fifty-eight were of the lymphogenous type, 58 of the myeloid, and 1 of the monocytic variety.

The enlarged spleen and ribs were irradiated in myelogenous leukemia. In most cases the total white count fell rapidly and continued to do so even after irradiation was stopped. For this reason it is wise to discontinue therapy when a level of 50,000 to 75,000 white cells has been reached. In lymphoid leukemia the various lymph node enlargements were irradiated as well as the liver and spleen if enlarged.

At some period in the disease a refractory stage usually appears, and, when it does, it is customary to use heavier filtration and a greater target skin distance. Total body irradiation may also be employed. It is the best policy not to treat leukemic patients in the absence of symptoms. In this way the appearance of the refractory stage may be postponed.—E. A. L.

SACHS, M. D. [Univ. of Oregon Med. Sch., Portland, Ore.] DANGERS AND USES OF RADIUM IN THE TREATMENT OF CARCINOMA OF THE UTERUS. West. J. Surg., 48:609-615. 1940.

Possible dangers associated with the intra-uterine insertion of radium for cancer of the cervix or fundus are likely to be materialized when treatment is undertaken by an inexperienced physician. Details of technic are described and the report contains drawings and roentgenograms.—M. J. E.

SALINGER, S. [Chicago, Ill.] RADIATION THERAPY FOR CARCINOMA OF THE LARYNX: OBSERVATIONS AFTER TWENTY YEARS. Arch. Otolaryng., 32:887-902. 1940.

This is a general discussion of the principles underlying surgical and radiotherapeutic methods currently employed in the treatment of laryngeal cancer.—M. J. E.

SCHMITZ, H. E., and J. F. SHEEHAN. [Loyola Univ. Sch. of Med. and Mercy Hosp., Chicago, Ill.] FIVE YEAR END-RESULTS IN CERVICAL CARCINOMA TREATED WITH RADIUM AND 800 KILOVOLT ROENTGEN RAYS. Am. J. Roentgenol., 45:229-234. 1941.

This is a review of the effects of supervoltage roentgen therapy on the skin, blood, mucous membranes, cancer cells, and tissues of the pelvis and a report of the 5-year end results in the primary cases treated with x-ray and radium.

The x-ray factors employed were 800 kv. maximum, 10 ma., filter equivalent to 10 mm. copper, a focal skin distance of 70 cm. and entrance fields varying in size from 300 to 400 sq. cm. Usually two fields were used and a dose of 4,000 to 4,500 r measured with backscatter delivered to each in a period of 28 days.

Desquamation and wet dermatitis appeared 28 days after treatment was started and had healed 3 weeks later. A mild leukopenia was commonly produced but there were no constant effects on the erythrocyte count. Microscopically the tumor cells showed progressive swelling of the cytoplasm and nucleus, loss of regularity of the tumor pattern, bizarre cell forms, multinucleated giant cells, and, later, abundant stroma.

Of the 26 primary cases treated with x-ray and 4,500 mgh. of radium, divided into 3 weekly doses, 12, or 46.1%, survived 5 years.—E. A. L.

SHARP, G. S. [Tumor Clinic, Huntington Memorial Hosp., Pasadena, Calif.] RADIUM AND X-RAY IN TREATMENT OF CANCER OF HEAD AND NECK. Arch. Phys. Therapy, 21:549-557. 1940.

Various combinations of radiotherapy and conservative surgical measures utilizable in the treatment of cancer of the lip and mouth are illustrated by 5 case reports. In the first patient a cancer of the lip was irradiated by means of a radium plaque. In 4 patients tumors of the buccal mucosa, tongue, or soft palate were eradicated with fractionated roentgen therapy and interstitial radium needles. In 2 instances irradiation was administered to metastatic nodes in the neck which were exposed surgically. With the single exception of an adenocarcinoma of the palate all tumors were squamous cell cancers. At the end of periods of 3 to 5 years the patients appeared tumor-free.—M. J. E.

SIMPSON, F. E., J. E. BREED, and J. S. THOMPSON. [Chicago, Ill.] CANCER OF THE TONGUE WITH A REPORT OF 40 CASES TREATED WITH LEAD RADON TUBULES. Illinois M. J., 78:16-23. 1940.

In operable and inoperable cases of cancer of the tongue the puncture method of employing radon offers the possibility of obtaining results comparable with those following glossectomy. The authors utilize permanently implanted tubules made of capillary lead tubing containing 5% antimony for the insertion of the radon. The tubules are 2 to 3 mm. in length and contain 0.5 mc. radon. No permanent injury has been observed as they become completely encysted in the tissues. A preliminary surface treatment with radon (250 mc. hrs.) is given. The needles are then inserted at distances of 1 cm. throughout the lesion and numerous weak radiating foci are preferred to several strong foci. The total dose does not exceed 30 to 35 mc. distributed in 60 to 70 tubules. A radium bomb is generally employed in treating metastases in the neck. The results in 40 unselected cases are given. Palpable nodes in the neck were present in 26. Of 12 patients cured for periods varying from 3 to 9 years, 5 had involvement of the cervical nodes. In addition 3 patients were tumor-free for 2 to 4 years, but died of intercurrent disease. Roentgenograms and photomicrographs are included.—M. J. E.

SOILAND, A. [Los Angeles Tumor Inst., Los Angeles, Calif.] THE PROGRESS OF RADIUM SINCE ITS EARLIEST THERAPEUTIC AVAILABILITY. Am. J. Roentgenol., 45:676-682. 1941.

This is a discussion in the development of the clinical use of radium and is accompanied by a brief review of the literature on the use of teleradium and ultra-short wave roentgen rays.—E. A. L.

STONE, R. S., J. H. LAWRENCE, and P. C. AEBERSOLD. [Univ. of California, San Francisco, Calif.] A PRELIMINARY REPORT ON THE USE OF FAST NEUTRONS IN THE TREATMENT OF MALIGNANT DISEASE. Radiology, 35:322-327. 1940.

Twenty-four patients with far advanced malignant disease of the face, neck, and upper chest were treated with fast neutrons. The amount given per treatment varied from 60 n to 275 n. Several of the patients were treated over more than one field but on different days, and some

of the fields were treated more than once, after the first effect wore off. In general, doses of 180 to 200 n administered to 10 x 10 cm. fields produced a moderate erythema which appeared between the 7th and 11th days, reached its peak on about the 21st day, gradually changed from erythema to pigmentation and left little residual change after a few months. There was some decrease in size of all of the primary tumors and in the metastases. The most promising results were obtained in the cervical metastases. Eight patients lived more than 1 year but all still had other tumors. It is emphasized that the treatments given were single erythema doses, not even skin doses, and were not fractionated.—E. A. L.

STONE, R. S., and J. M. ROBINSON. [Univ. of California Med. Sch., San Francisco, Calif.] ROENTGEN IRRADIATION OF THE PELVIS IN CARCINOMA OF THE CERVIX UTERI. Radiology, 36:521-533. 1941.

The roentgen ray beam is directed toward the probable regions of extension and metastasis which are beyond the reach of radium applied to the cervix and uterine canal. There are 3 main sets of lymph channels from the region of the cervix. The first set drains to the nodes adjacent to the external iliac artery. The second group drains to the hypogastric nodes near the bifurcation of the iliac arteries. The third drains posteriorly through the uterosacral ligaments to the sacral nodes beneath the sacral promontory. All of these areas as well as the upper portion of the vagina must be included in the radiation fields. Routinely 2 anterior and 2 posterior fields are used, each usually being 10 cm. wide and 15 cm. long. Right and left lateral fields are added if the patient is more than 20 cm. thick. A strip at least 2 cm. wide is left down the midline anteriorly and posteriorly. The inferior margin of the anterior fields is at the level of the upper limit of the vulvae. The posterior fields are directly opposite. All fields should be checked by films made with the therapy apparatus. When 200 kv. x-rays are used the daily dose is 200 r in air each to an anterior and to the corresponding posterior port. Treatment should be carried to toleration which may be as high as 2,300 or even 2,700 r to each port. When 1,000 kv. x-rays are used the daily dose in air is 300 to 325 r and the total dose is between 3,250 and 3,750 r.—E. A. L.

TEAHAN, R. W. [Jeanes Hosp., Philadelphia, Penn.] THE TREATMENT OF CARCINOMA OF THE BREAST BY INTERSTITIAL RADIATION. Am. J. Roentgenol., 45:567-588. 1941.

This is a review of the literature and a report of 68 cases treated in this manner. Needles 60 mm. long, containing 3 mgm. of radium, and 44 mm. long, containing 2 mgm., are used. All have a wall thickness of 0.8 mm. of platinum. Two rows of the longer needles are placed 1.5 cm. apart beneath the breast and upward into the anterior wall of the axilla. Additional needles are placed in each of the other axillary walls. The shorter needles are used in the infra- and supraclavicular fossae, in the 4 upper intercostal spaces, and in the power part of the breast between the implanted area and the epigastrium. The total dose has varied between 848 and 32,329 mgm. hr. The healing of the radiation reaction is complete in 4 to 6 weeks. Differentiation between residual carcinoma and postradiation fibrosis in the breast and in the axilla is difficult. Of the 68 patients to treated, 37 are now living.—E. A. L.

WALKER, A. E., C. H. JESSICO, and A. W. MARCOVICH. (Univ. of Chicago, Chicago, Ill.] THE MYELOGRAPHIC DIAGNOSIS OF INTRAMEDULLARY SPINAL CORD TUMORS. Am. J. Roentgenol., 45:321-331. 1941.

This is a review of a series of intramedullary spinal cord tumors in order to show myelographic criteria for differentiating intra- and extramedullary spinal cord neoplasms. The criteria for tumefaction in the spinal cord are a partial block, lateral displacement of the lipiodol, and extension of the displaced lipiodol in beaded columns for several segments of the spinal cord.—E. A. L.

WASSON, W. W., J. S. BOUSLOG, and A. P. JACKSON, JR. [Denver, Colo.] INTRA-ORIFICIAL ROENTGEN THERAPY. Radiology, 35:676-679. 1940.

The use of cylinders, with obturators, an eye piece and air compression, for x-ray radiation of tumors of the cervix, rectum, and bladder is described.—E. A. L.

WIDMANN, B. P. [Philadelphia Gen. Hosp., Philadelphia, Penn.] RADIATION THERAPY IN CANCER OF THE SKIN. Am. J. Roentgenol., 45:382-394. 1941.

A method of low voltage (90 to 135 kv.) roentgen ray therapy for treatment of skin cancer is presented which is based on the thickness or approximate bulk of the tumor. For lesions that are elevated less than 0.5 cm. above the level of the surrounding normal skin three treatments of 1,000 r each are given on alternate days. A margin of surrounding normal skin from 0.5 to 1.5 cm. wide for tumors 1 to 5 cm. in diameter is included in the irradiated field. Tumors that are elevated more than 0.5 cm. are given 3 treatments of 1,500 r each on alternate days. A liberal margin of normal skin is included. With each technic 1,000 r additional can be given 2 to 3 weeks later to sharply localized fields suspicious of residual tumor.—E. A. L.

WILKINS, G. C. [Elliot Hosp., Manchester, N. H.] CARCINOMA OF THE CERVIX. A REVIEW OF 200 CASES TREATED WITH RADIUM. New England J. Med., 224:414-415. 1941.

The author treated 200 microscopically confirmed cases of carcinoma of the cervix between 1920 and 1934 by radium alone. The 5 year survival percentages for this group, Stages I-IV (League of Nations), were 63, 52, 35, and 0. The technic is described.—A. M.

WOODARD, H. Q., and N. L. HIGINBOTHAM. [Memorial Hosp., New York, N. Y.] SERUM AND TISSUE PHOSPHATASE DETERMINATIONS AS AN AID IN EVALUATING THE RADIATION THERAPY OF BONE TUMORS. J. A. M. A., 116:1621-1627. 1941.

Examination of tissue from untreated neoplasms of bone shows that benign osteochondromas and giant cell tumors produce little phosphatase. Some osteogenic sarcomas produce little phosphatase, some produce abundant phosphatase which does not enter the circulation readily, but some produce abundant phosphatase which enters the circulation readily and can be measured in the serum. The phosphatase-producing mechanism of most of these tumors is inactivated by radiation therapy when the tissue dose equals or exceeds 4,000 r. Smaller doses cause only irregular or incomplete inactivation. In patients with osteogenic sarcoma having elevated serum phosphatase

values, the change in phosphatase affords a prompt indication of the effect of radiation therapy. Radioactive phosphorus given by mouth localizes in the portions of tissue of osteogenic sarcoma which contain the most phosphatase. Serum phosphatase determinations indicate the effectiveness of radiation therapy of metastatic tumors of bone only after sufficient time has elapsed for some healing to take place. Determinations of the alkaline and acid phosphatase in the serum of patients with carcinoma of the prostate metastatic to bone make it possible to follow the activity of both the metastatic tumor and the regeneration of bone.—H. G. W.

Skin and Subcutaneous Tissues

ANDERSON, W. A. D. [Univ. of Tennessee Coll. of Med., Memphis, Tenn.] DISEASES OF THE AMERICAN NEGRO. 1. MELANOMA. Surgery, 9:425-432. 1941.

The author's review of the literature shows melanoma to be far from rare among negroes. He presents 10 cases from the John Gaston Hospital in Memphis. Twelve cases occurred in white patients during this period, although the latter only represented 22% of the patients. Melanoma appears to occur more frequently in the white race 4.2:1) but its microscopic appearance and clinical behavior is the same in both races. Eight photographs and a bibliography of 18 papers are included.—A. M.

BECKER, A. [Path. Inst. der Univ. Giessen, Germany] ÜBER EIN LUPUSSARKOM. [LUPUS SARCOMA.] Virchows Arch. f. path. Anat., 306:518-525. 1940.

Neoplastic alteration on the basis of lupus vulgaris is commonly of epithelial origin and associated with long-standing roentgen therapy of the tuberculous process. A case is reported of spindle cell sarcoma which developed in a nonirradiated lupus lesion of the gluteal region. The patient died of pulmonary metastases. Facial lupus had reacted well to irradiation therapy.—M. J. E.

HATCHETTE, S. [Lake Charles, La.] EARLY RESULTS IN THE TREATMENT OF CANCER OF THE SKIN BY THE METHOD OF CHAOUL. New Orleans M. & S. J., 93:509-513. 1941.

The x-radiation delivered by the Chaoul machine would seem to be almost ideal for the treatment of malignant lesions of the skin. The distribution of dosage within the tissues seems to be identical with that of the gamma rays of radium.—H. G. W.

HERSPERGER, W. G., and W. NEILL. [Baltimore, Md.] MALIGNANT MELANOMA. REPORT OF A CASE RECURRING AFTER FOURTEEN YEARS. Am. J. Surg., 52: 111-114. 1941.

Case report of melanoma of the skin, with references to similar cases of long latency reported in the literature.—H. G. W.

JESSUP, D. S. D. [New York Post-Graduate Med. Sch. and Hosp., Columbia Univ., New York, N. Y.] XERODERMA PIGMENTOSUM WITH TUMOR FORMATION. Am. J. Cancer, 40:324-328. 1940.

A case of xeroderma pigmentosum in a 12-year-old boy is described. The case has been under observation for 8 years and numerous squamous cell carcinomas and one basal cell epithelioma have developed.—L. L. W.

JOHNSON, G. S. [Vanderbilt Univ., Nashville, Tenn.] THE TREATMENT OF CARCINOMA OF THE LIP. J. Tennessee M. A., 33:268-274. 1940.

Cancer of the lower lip rarely spreads beyond the neck, and treatment should be started before the regional nodes are involved. In cases of early cancer surgery is simple and effective. Small tumors may be extirpated with a cautery knife, larger types by a rectangular excision followed by plastic repair. Radiation appears superior for advanced tumors. Six cases are described.—M. J. E.

KIRBY, D. B. [New York, N. Y.] NEUROMYOARTERIAL GLOMUS TUMOR IN THE EYELID. Arch. Ophth., 25:228-237. 1941.

Glomus tumors are small benign painful lesions in the skin, occurring also under the fingernails. The author records the first growth of this type in the eyelid. It had appeared 8 years previously, was excised, but recurred. A wide excision was then employed and a cure achieved. Although the normal glomus has not been identified in the eyelid, the histologic character of the excised mass was typical of a glomus tumor.—M. J. E.

PHILLIPS, C. [Scott and White Clinic, Temple, Tex.] OBSERVATIONS BASED UPON THE STUDY OF 1,434 SKIN CANCERS. Virginia M. Monthly, 67:400-406. 1940.

The frequency of various forms of histologically verified skin cancers observed during 18 years in a large clinic in Texas is analyzed. The material was from 1,182 patients. Multiple tumors varying from 2 to 12 in number were relatively frequent. Skin neoplasia were uncommon in Negroes and Mexicans, while in white individuals blondes appeared to show a predilection. Histologically there were 647 basal cell cancers, 647 squamous cell epitheliomas, and 140 melanomas. From previous experience in a more northerly climate the author is of the opinion that the incidence of skin cancer is higher in southern regions, especially when prolonged exposure to the sun occurs in a large proportion of the population engaged in agricultural pursuits.—M. J. E.

SCHREK, R., and O. GATES. [Pondville Hosp., Wrentham, Mass., and Collis P. Huntington Memorial Hosp., Boston, Mass.] CUTANEOUS CARCINOMA. Arch. Path., 31:411-443. 1941.

I. A statistical analysis with respect to the duration and size of the tumors and the age of the patients at onset and at biopsy of the tumor. An analysis was made of data obtained in 581 cases of cutaneous carcinoma. The median duration of basal cell carcinomas (3.5 years) was much greater than that of epidermoid carcinoma (1.2 years). The median size of the two types of tumor was, however, the same (1.9 cm.), indicating that the size, not the duration of the tumor, prompted the average patient to seek hospital treatment. The basal cell tumors developed in a much younger group of patients than the epidermoid carcinoma, the median age for the former being 57.3 years as against 66.2 years for the latter.

II. A statistical analysis with respect to measures of innate and clinical malignancy. Tumors have two distinct types of malignancy, innate and clinical. The innate malignancy is the degree of deviation of the tumor from the prototype. Clinical malignancy is the hazard of the tumor to the life and health of the patient. Most of the measures of innate and clinical malignancy agree that

epidermoid carcinoma is about 3 times as malignant as basal cell carcinoma, but in spite of its low malignancy the basal cell type recurs in as high a percentage of cases as the epidermoid type.

III. A statistical analysis with respect to site, sex, and pre-existing scars. Epidermoid carcinoma has a marked predisposition for the ears, hands, and upper part of the face, whereas the basal cell carcinoma prefers the upper part of the face, the nose, and the ears, in order of frequency. Both types occur with higher frequency in males, especially on the ears. Eighteen % developed in pre-existing scars. About 16% of the epidermoid carcinomas metastasized to regional lymph nodes, the site of the tumor being apparently of no influence.—H. G. W.

SHELDON, W. H. [Free Hosp. for Women, Brookline, Mass.] THE MYOEPITHELIUM IN SWEAT GLAND TUMORS. Arch. Path., 31:326-337. 1941.

Of 10 sweat gland tumors, 3 showed neoplastic proliferation of the myoepithelium, 1 benign and 2 malignant. The origin of the myoepithelium is discussed, and the conclusion favored that there is probably a borderline region between ectoderm and mesoderm where the cells are endowed with the potentialities of both tissues, and from such cells the myoepithelial tumors arise.—H. G. W.

Female Genital Tract

CURTIS, A. H. [Northwestern Univ. Med. Sch., Chicago, Ill.] MANAGEMENT OF CARCINOMA OF CERVIX. Wisconsin M. J., 39:728-731. 1940.

This is a general discussion of the technic of radiotherapy of cervical cancer.—M. J. E.

HALL, N. D. [Phoenix, Ariz.] CARCINOMA OF THE CERVIX IN THE FIRST THREE DECADES OF LIFE. Canad. M. A. J., 43:362-365. 1940.

The author analyzes 57 cases of histologically proved cervical cancer in women 30 years of age or younger observed at the Barnard Free Skin and Cancer Hospital, St. Louis, Mo. The youngest patient was 20 years of age. Early marriage and pregnancy are contributing factors in the etiology of the condition, as 21 patients in this series had one or more pregnancies before attaining the age of 20. Treatment does not differ from that employed in older age groups, but the results are poorer. Of the 52 patients traced 31 were dead and 21 survived after intervals varying from 1 month to 5 years.—M. J. E.

SCHMITZ, H. E., and P. A. NELSON. [Loyola Univ. Clinics, Chicago, Ill.] CLINICAL CLASSIFICATION OF CANCER OF THE CERVIX. Am. J. Roentgenol., 45:395-402. 1941.

A standard basis for clinical classification of carcinoma of the cervix is offered because of its practical adaptability. In group I the disease is clearly localized to the cervix. In group II the growth is doubtfully localized and there is impeded mobility of the uterus. In group III there is invasion of the parametria or lymph nodes but the entire mass is movable. In group IV there is either a frozen pelvis, local dissemination to the bladder, vagina, or rectum, or distant metastases.—E. A. L.

STACY, W. T., and F. G. THOMPSON. [St. Joseph, Mo.] CERVICAL CARCINOMA ASSOCIATED WITH PREGNANCY AT FULL TERM. J. Missouri M. A., 38:82-84. 1941.

A diagnosis of cervical cancer was first established in a woman of 30 at full term when unexplainable vaginal bleeding prompted a speculum examination. The child was delivered by cesarean section and a panhysterectomy performed. Subsequently a massive pelvic recurrence developed associated with vesicovaginal and rectovaginal fistulae, and the patient died 13 months after the first operation.—M. J. E.

BACKUS, G. R., and E. P. GRIFFIN, JR. [Hurley Hosp., Flint, Mich.] PRIMARY CHORIONEPITHELIOMA OF THE OVARY. Am. J. Clin. Path., 11:252-257. 1941.

Report of a case in a 13-year-old virgin, with fatal outcome.—H. G. W.

SMITH, E. C., and D. W. GOLDMAN. [Sch. of Med., Louisiana State Univ., New Orleans, La.] CHORIONEPITHELIOMA. South. M. J., 34:486-493. 1941.

A study of 15 suspected cases, of which 10 were verified, with special reference to diagnosis. The disease was infrequent in colored as compared with white women. The mortality was not influenced by x-ray or radium.—H. G. W.

BARON, H. A. [Jewish Gen. Hosp., Montreal, Canada] PRIMARY CARCINOMA OF THE FALLOPIAN TUBE. Canad. M. A. J., 43:118-121. 1940.

Report of an inoperable case of adenocarcinoma of the Fallopian tube.—M. J. E.

MALLORY, T. B., Editor. [Boston, Mass.] CASE RECORDS OF THE MASSACHUSETTS GENERAL HOSPITAL. CASE 27101. New England J. Med., 224:427-428. 1941.

A case of primary adenocarcinoma of the fallopian tube is presented.—A. M.

WALKER, M. A., and H. H. HESSER. [Univ. of Kansas Sch. of Med., Kansas City, Kan.] CARCINOMA OF THE FALLOPIAN TUBE. REPORT OF TWO CASES. J. Kansas M. Soc., 41:469. 1940.

A tumor resection was attempted in 2 patients with primary cancer of the uterine tube. The first died postoperatively, the second after 7 months with peritoneal dissemination of the growth.—M. J. E.

GABRILOVE, J. L. [Beth Israel Hosp., New York, N. Y.] CARCINOID IN STOMACH TISSUE WITHIN AN OVARIAN DERMOID. Arch. Path., 31:508-509. 1941.

A case is reported of a carcinoid arising in stomach tissue which formed part of an ovarian dermoid. This is the 3rd case of carcinoid in an ovarian dermoid and the 14th case of a carcinoid in stomach tissue.—H. G. W.

JONES, F. H. [White Memorial Hosp., Los Angeles, Calif.] PAPILLARY CYSTADENOCARCINOMA OF THE OVARY. Am. J. Surg., 52:246-259. 1941.

Analysis of the records of 30 cases, 6 of which survived 5 years or more.—H. G. W.

LORBER, H., and M. VESELL. [302 W. 90th St., New York, N. Y.] ESTROGENIC DETERMINATIONS IN A CASE OF DISGERMINOMA OVARII COMPLICATED BY PREGNANCY. Endocrinology, 27:157-159. 1940.

A case report is given of disgerminoma ovarii in a 23-year-old woman who was 3 months pregnant. The right ovary which contained the tumor (13 x 12 x 9 cm.) and the corpora lutea of pregnancy were removed by surgery. Gestation was not disturbed and the patient made an uneventful recovery. Estrogen secretion for the 10 days following operation increased only from 3,000 to 3,5000 m.u. per liter of urine indicating that the disgerminoma ovarii did not influence the hormonal status of the patient. —C. A. P.

MacFEE, W. F. [St. Luke's Hosp., New York, N. Y.] BENIGN TUMORS OF THE OVARY ASSOCIATED WITH ASCITES AND PLEURAL EFFUSION. REPORT OF A CASE OF MULTILOCULAR CYSTADENOMA. Ann. Surg., 113: 549-555. 1941.

The presence of pleural effusion and ascites complicating benign ovarian tumors was described as early as 1879 and at intervals since. Meigs recently popularized the syndrome. The author reports the removal of a 17 pound benign multilocular cystadenoma associated with a massive right pleural effusion. No fluid had recurred in over 2 years since operation. Five photographs are given, and a bibliography of 17 papers.—A. M.

NOVAK, E. [Baltimore, Md.] OVARIAN TUMORS OF ENDOCRINE NATURE. J. A. M. A., 116:947-950. 1941.

A review, to be published as a chapter in book form as "Glandular Physiology and Therapy." It deals with the endocrine activity of granulosa cell carcinoma, thecoma, luteoma, arrhenoblastoma, adrenal ovarian tumors, and thyroid tumors of the ovary.—H. G. W.

TUTA, J. A., and J. E. SIEBEL. [Grant Hosp., Chicago, Ill.] MALIGNANT MESONEPHROMA OF THE OVARY. Arch. Path., 31:386-390. 1941.

A malignant cystic ovarian tumor has been classified, because of its characteristic histologic structure, as belonging in a group probably of mesonephric origin. A comparison of the relatively benign primary tumor and the more malignant recurrence showed that both had a common characteristic histologic structure.—H. G. W.

WALTER, R. I., A. L. BACHMAN, and W. HARRIS. [Mount Sinai Hosp., New York, N. Y.] THE TREATMENT OF CARCINOMA OF THE OVARY. Am. J. Roentgenol., 45: 403-411. 1941.

The literature is reviewed, and a series of 124 cases with carcinoma of the ovary seen from 1928-35 is presented. Operative removal alone produced a corrected 5-year survival rate of 6.77% while surgery plus adequate postoperative x-ray therapy produced a survival rate of 33.3%.—E. A. L.

WATTS, R. M., and F. L. ADAIR. [Univ. of Chicago and Chicago Lying-in Hosp., Chicago, Ill.] OCCURRENCE OF ESTROGENIC HORMONE IN OVARIAN CYSTS. Cancer Research, 1:638-648. 1941.

An attempt has been made to correlate the occurrence of estrogenic hormone in ovarian cyst fluids with the morphology of the cyst. The study included 189 benign and 23 malignant ovarian cysts.

Estrogenic hormone was found chiefly in cysts arising from ovarian structures. In 192 cyst fluids from benign tumors solely of one type of cyst the percentages of the different types which showed hormone were: follicle, 76.5; corpus luteum, 46.2; simple serous, 20.7; papillary serous, 10.0; pseudomucinous, 8.3; dermoid, none; miscellaneous, 33.3. In 102 cyst fluids obtained from benign tumors composed of more than one type of cyst, bilateral tumors and those occurring in patients with histories of previous ovarian disease, the percentages of positive fluids were: follicle, 47.1; corpus luteum, 50.0; simple serous, 32.0; papillary serous, 18.2; pseudomucinous, 21.7; dermoid, none; miscellaneous, 50.0.

When estrogenic hormone was present the concentration r.u. per cc. varied with the different types of cyst fluids: follicle, 0.07-33; corpus luteum, 0.12-2; simple serous, 0.005-0.2; papillary serous and pseudomucinous usually less than 0.005; dermoids, none.

Three of the 55 fluids of malignant ovarian cysts contained estrogenic hormone at 0.01, 0.013, and 0.03 r.u. per cc.

In a control series, 28 cyst fluids from nonovarian genital tumors and 36 ascitic fluids were negative for estrogenic hormone, with the exception of one parovarian cyst.—Authors' abstract.

CROSSEN, H. S. [Wash. Univ. and Barnes Hosp., St. Louis, Mo.] ADVANCES IN THE TREATMENT OF CANCER OF THE CORPUS UTERI. J. Missouri M. A., 37:376-386. 1940.

The author advises preoperative intra-uterine application of radium in operable cases of cancer of the uterine body and radium alone in the inoperable cases. The technic of tandem and supplementary disposition of radium capsules with the aid of a wire distributor is described in detail.—M. J. E.

GREENBLATT, R. B., and E. A. WILCOX. [Univ. of Georgia Sch. of Med., Atlanta, Ga.] HORMONAL THERAPY OF FIBROMYOMAS OF THE UTERUS. South. Surgeon, 10:339-346. 1941.

The authors employed androgenic hormones in the treatment of bleeding in patients with fibromyoma of the uterus, on the basis that this symptom is a result of an associated endocrine disturbance and not primarily dependent on the presence of a mass. Six patients with tumors 1 cm. in diameter or smaller received parenterally 20 to 75 mgm. testosterone propionate monthly or methyl testosterone orally. In 10 with larger fibroids pellets of the former substance in doses of 25 to 145 mgm. were inserted under the fascia lata of the thigh. Hemorrhage was controlled during the period of action of the male hormone, but recurred when therapy was omitted. A prolonged effect was produced by the slowly absorbed pellets. The androgens had no adverse influence on the feminine habitus.—M. J. E.

LEVI, A. A. Tufts Med. Sch., Boston, Mass.] PEDUNCULATED ENDOMETRIAL CYST OF THE UTERUS. New England J. Med., 224:156-158. 1941.

This paper is a report of a case of pedunculated endometrial cyst, removed vaginally. Such cysts are very rare. Operation on this patient showed endometriosis of the uterine wall and a large ovarian dermoid cyst.—A. M.

LIEBOW, A. A., and R. TENNANT. [Yale Univ., Sch. of Med., New Haven, Conn.] MESODERMAL MIXED TUMORS OF THE BODY OF THE UTERUS. Am. J. Path., 17:1-30. 1941.

The authors reviewed 68 cases of mesodermal mixed tumors of the body of the uterus (65 from the literature and 3 of their own): almost all occurred in women between the ages of 45 and 65; most of the tumors were polypoid and arose from the cornua and the posterior wall; histologically they were composed predominantly of cartilage or striated muscle but in addition contained (in order of frequency) undifferentiated sarcomatous elements, epithelium, giant cells, smooth muscle, bone or osteoid, fat, nervous tissue, and endothelium. From special histological and tissue culture studies the authors concluded that the pathogenesis depended on multipotential anlagen, not metaplasia.

Clinically, sanguineous discharge and abdominal pain were characteristic. Radical hysterectomy was performed in most cases, but nevertheless, death occurred in 19 of 28 cases which had been followed about 52 weeks after the onset of symptoms. Local metastases were usually found.—H. B.

MALLORY, T. B., Editor. [Boston, Mass.] CASE RECORDS OF THE MASSACHUSETTS GENERAL HOSPITAL. CASE 27072. New England J. Med., 224:291-293. 1941.

A report of a case of polyploid adenocarcinoma of the uterus, grade I. The patient had had abnormal vaginal bleeding for 6 years prior to admission.—A. M.

MILLER, J. R. [Hartford, Conn.] TREATMENT PROCEDURES FOR CANCER OF THE UTERUS ADVISED BY THE CONNECTICUT TUMOR CLINICS. Connecticut M. J., 4:16-19. 1940.

The suggested programs for the treatment of carcinoma of the cervix and of the body of the uterus are presented in some detail in order to establish uniformity in methods and to evaluate their results.—A. DeB.

PATTERSON, D. C., W. A. GEER, and E. M. STRAYER. [Bridgeport Hosp., Bridgeport, Conn.] A CASE OF INGUINAL ENDOMETRIOMA. Arch. Surg., 42:577-580. 1941.

A case of inguinal endometrioma which became more painful and larger with the menses is reported.—G. De B.

PLASS, E. D., [State Univ. of Iowa Col. of Med., Iowa City, Iowa] THE FIBROID UTERUS. J. Missouri M. A., 38:1-3. 1941.

General clinical remarks.—M. J. E.

SMITH, F. R. [New York, N. Y.] SARCOMA OF THE UTERUS. New York State J. Med., 41:681-685. 1941.

Twenty-four cases are tabulated. Symptoms are atypical and a correct diagnosis is rarely established in early stages. Associated fibroids were present in 14 patients. Hysterectomy and radiotherapy are advised, but the growths are not radiosensitive. No patients survived more than 4 years after the first hospitalization.—M. J. E.

STEVENSON, C. A. [Scott and White Clinic, Temple, Tex.] ROENTGEN DIAGNOSIS OF CALCIFIED PELVIC TUMORS. Tex. State J. Med., 36:422-426. 1940.

Roentgenograms frequently disclose calcium deposition in tumors of the internal genitalia in females. A small percentage of uterine fibroids contain circumscribed opaque, calcified areas. Of 52 ovarian dermoids 26 had areas of bone or imperfectly formed teeth. Noncalcified dermoids may show roentgenographically a less dense inner zone formed of accumulated secretion contrasted with a more dense outer rim of squamous epithelium. Lime deposits were also observed in a single fibroma and cystadenoma of the ovary.—M. J. E.

TIGERT, H. M. [Nashville, Tenn.] CANCER OF THE UTERUS. Texas State J. Med., 36:675-675. 1941.

Clinical aspects of the problem are reviewed.—M. J. E.

CROSBIE, W. G. [Toronto Gen. Hosp., Toronto, Canada] CARCINOMA OF THE VULVA. Canad. M. A. J., 43:439-444. 1940.

Operable cases of cancer of the vulva are best treated by radical vulvectomy combined with bilateral extirpation of the lymph nodes of Scarpa's triangle. The advantage of this technic over simple vulvectomy is illustrated by 2 groups of patients. Of 16 in the former group 13 had survived for periods up to 5 years, as contrasted with 5 survivals in the latter group for approximately similar periods. Irradiation alone or combined with surgery may give satisfactory results in some cases.—M. J. E.

HELD, E. [l'Univ. de Genève, Geneva, Switzerland] CONTRIBUTION À L'ÉTUDE DU TRAITEMENT DU CANCER DE LA VULVE. [TREATMENT OF CANCER OF THE VULVA.] Monatschr. f. Geburtsh. u. Gynäk., 111:195-199. 1940.

Electrocoagulation is recommended as the treatment of choice in elderly patients with large cancers of the vulva. Three cases are described in which this procedure produced an excellent temporary local result.—M. J. E.

Male Genital Tract

HIGGINS, W. H. [Johns Hopkins Med. Sch., Baltimore, Md.] UNUSUAL URETERAL EXTENSION OF PROSTATIC CARCINOMA. Bull. Johns Hopkins Hosp., 68:337-346. 1941.

Report of a case, with emphasis on the possibility that cases reported as metastasis to the ureter from the pelvic organs may have been by direct extension.—H. G. W.

KULVIN, M. M. [U. S. Veterans Facility, Hines, Ill.] METASTATIC CARCINOMA OF CHOROID WITH PRIMARY FOCUS IN PROSTATE GLAND. Am. J. Ophth., 23:892-899. 1940.

A case report of a cancer of the prostate which metastasized to the pelvis, vertebral column, ribs, and choroid of the right eye. Radiotherapy had been administered to the primary tumor.—M. J. E.

THOMPSON, G. J. [Mayo Clinic, Rochester, Minn.] CARCINOMA OF THE PROSTATE: ITS CONSERVATIVE SURGICAL TREATMENT. South. Surgeon, 10:271-278. 1941.

Cancer of the prostate is usually not diagnosed until some degree of urinary obstruction is present. At that time the malignant process has extended sufficiently through the lymphatic vessels to make complete surgical removal impossible. Transurethral resection of the obstructing tissue and postoperative roentgen therapy are preferred to the more radical prostatectomy. The operative mortality is low and the results are encouraging. Of the 253 patients treated 5 or more years previously with available follow-up records, 74% survived 1 year, 46% 2 years, 31% 3 years, 17% 4 years, and 8.7% 5 years or longer.—M. J. E.

NEAL, M. P., and J. F. JOLLEY. [Univ. of Missouri Sch. of Med., Columbia, Mo.] SPERMATIC CORD TUMORS. J. A. M. A., 116:1218-1220. 1941.

A report of a case of fibromyxolipoma of the spermatic cord is added to the 27 reports listed in the literature, and the total of 247 tumors of all sorts of the spermatic cord.—H. G. W.

BARRINGER, B. S., and D. EARL. [Memorial Hosp., New York, N. Y.] TERATOMA TESTIS. Surg., Gynec. & Obst., 72:591-600. 1941.

Teratoma testis is unique among malignant tumors in that a majority have an absolute x-radiation sensitivity, so that by means of high voltage roentgen therapy the present rate of 5 year controls is 30%. The dissection of the retroperitoneal tissues is superfluous, for if metastases are present the procedure is inadequate. A study of 37 cases with autopsy records shows that finally metastases are both blood and lymph borne as a rule, with involvement of the lungs in 78%; and where the lung is involved the

liver was involved in 75% and the mediastinal nodes in 55%. If there is present a left supraclavicular node there is probably a metastatic chain of nodes along the course of the thoracic duct. The genito-urinary tract was involved in 24%. Abdominal cryptorchidism with teratoma occurred 4 times, or 10.8%. There were 10 cases of embryonal carcinoma with lymphoid stroma, and 9 cases of embryonal carcinoma, or 51.3% of these most radiosensitive types.—H. G. W.

HUFFMAN, L. F. [Cleveland, Ohio] INTERSTITIAL CELL TUMOR OF THE TESTICLE. J. Urol., 45:692-697. 1941.

The 13th case of interstitial cell tumor is reported, in a boy of 6 who showed gynecomastia and slight precocious development of secondary sex changes.—H. G. W.

ORMOND, J. K., and C. L. PRINCE. [Henry Ford Hosp., Detroit, Mich.] MALIGNANT TUMORS OF THE TESTICLE. J. Urol., 45:685-691. 1941.

Report of 21 cases, only one of which was in a cryptorchid. One was a chorionepithelioma, which followed trauma. Most of the cases were treated by orchidectomy followed by x-ray therapy, and most of the cases without demonstrable metastases at the time of operation are living and well.—H. G. W.

Urinary System—Male and Female

BUGBEE, H. G. [New York, N. Y.] TUMORS OF THE BLADDER. New York State J. Med., 41:1085-1088. 1941.

The excellent temporary results which may be achieved in cases of advanced cancer of the bladder by a combination of direct local destruction of a sloughing tumor and radiotherapy, or irradiation alone are illustrated by 2 case reports. In the first case roentgen therapy was administered following fulguration of the growth and excision of the superficial portions with the resectoscope. In the second, roentgen therapy was employed for a tumor which recurred rapidly following removal by diathermy. Both patients were symptomatically relieved and cystoscopic examination disclosed complete disappearance of the tumors several months after treatment was concluded.—M. J. E.

FITZGERALD, J. S. [Utica, N. Y.] A CASE OF PAPILLARY CARCINOMA IN A HORSESHOE KIDNEY. New York State J. Med., 41:1081-1084. 1941.

The congenital anomaly was not discovered preoperatively. The carcinomatous kidney was removed and the patient appeared in satisfactory condition 9 months later.—M. J. E.

FROUG, C. [Dayton, Ohio] LIPOSARCOMA OF THE KIDNEY. J. Urol., 45:290-295. 1941.

A review of the 6 cases of liposarcoma of the kidney with addition of 1 case, 4 of them being associated with tuberous sclerosis.—H. G. W.

PUENTE DUANY, N. [Univ. of Havana, Havana, Cuba] LIMFOSARCOMA Y LIMFOMATOSIS DE LOS RIÑONES. [LYMPHOSARCOMA AND LYMPHOSARCOMATOSIS OF THE KIDNEYS.] Rev. de med. trop. y parastiol., bacteriol. clín. y lab., 6:117-156, 213-251, 303-326. 1941.

In this monographic publication the author admits the possibility that some lymphosarcomas of the kidneys are primary and not metastasic, and classifies the lymphoid tumors of these organs as primary and secondary; the latter are divided into nodular and diffuse forms. The diffuse forms are further divided into unilateral and bilateral forms, and in the bilateral the author lists the tumoral, the leukemic, and the aleukemic types. The most common sort of neoplasm is the nodular metastasic, either simple or multiple, and affecting one or both organs. These lesions are without symptoms and are diagnosed only at autopsy. The diffuse metastasic form invades a part or the entire organ or may also be unilateral or bilateral. In the former case, despite the diffuse character of the lesion, only the capsule or the cortex is involved, and the author suggests the term "capsular" for them. He also emphasizes the importance of the bilateral diffuse sarcomatosis because of its greater anatomical and clinical interest. This condition is extremely rare—only 15 cases being recorded in the literature—and shows characteristic features. It has been given different names such as round cell infiltrating sarcoma, diffuse lymphomatosis, pseudoleukemia, etc. The disease occurs mostly in children, and is much more frequent in males than in females; it is always fatal. The patients often show signs of localized acute or subacute leukemia, but leukemic changes do not occur in the blood. In these cases the disease may run its entire course in 4 or 5 weeks. Other patients show typical symptoms of lymphosarcoma of the mediastinum, mesentery, digestive tract, etc. or a localized tumor, and in addition show bilateral involvement of the kidneys. The lesion can be diagnosed either clinically or at surgical intervention. The kidneys are much hypertrophied—4 to 5 times the normal size—but keep their normal shape. The capsule is much thickened but not adherent, and the parenchyma becomes discolored, with or without hemorrhage. Microscopic examination shows heavy infiltration by lymphoid like cells with subsequent necrosis of the renal parenchyma. The author thinks that the disease is an intermediate between typical lymphosarcoma and pseudoleukemia, and is most probably induced by a filterable virus. Fifteen cases of diffuse bilateral lymphomatosis are described, some in detail.—M. D-R.

WATSON, E. M., and C. C. HERGER. [New York State Inst. for the Study of Malignant Diseases, Buffalo, N. Y.] CARCINOMA OF THE BLADDER: A CORRELATION OF THE PATHOLOGICAL AND CLINICAL DATA AS A BASIS FOR TREATMENT. J. Urol., 45:331-336. 1941.

The results and methods of treatment of 445 cases of bladder tumor are presented. An attempt is made to correlate the clinical and pathological findings to establish a basis for choice of treatment. Statistics are presented in two 5-year groups. Twenty eight % of the patients with papillary tumors in the first group remained well over 5 years, while 12% of those with solid infiltrating tumors remained well over 5 years. In the second 5-year period disappearance of papillary tumors was obtained in 43% of cases for periods from 1 to 4 years, but in the non-papillary infiltrating group only 10% were alive and well for periods from 1 to 4 years.—H. G. W.